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Editor
ROY WALDO MINER

VIRUS AND RICKETTSIAL CLASSIFICATION AND NOMENCLATURE*

Conference Chairman SIR MACFARLANE BURNET

Consulting Editor HILARY KOPROWSKI

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VIRUS CLASSIFICATION AND NOMENCLATURE

By Sir MacFarlane Burnet
The Walter and Eliza Hall Institute for Medical Research Melbourne Australia

Probably there is no difference of opinion amongst virologists that were adequate knowledge of the interrelationships of viruses available an accepted and workable nomenclature would be a desirable convenience The same arguments that have been used in regard to bacterial nomenclature are also applicable to the virus problem A worker actively studying a particular group of viruses is usually quite happy to go on using the names that in one way or another become attached to his virus strains He may feel quite at home with Columbia SK MM Mengo and encephalomyocarditis viruses *and realize that they are all closely related forms For workers in other virus* fields for post graduate students or public health officers for anyone in short who wishes to know the significance of virus investigations without making a whole time study of virology the position would be much easier if a name as expressive as *Mycobacterium* or *Clostridium* were available to cover that particular group of viruses and provoke an immediate mental picture of a certain complex of practically demonstrable qualities An accepted nomenclature is still more desirable when the same virus is discussed in several different languages I need not labor this point nor does it seem necessary to look beyond the Linnean binomial system for the form of the names that might eventually be suggested The general acceptance of a binomial nomenclature for bacteria and other asexual forms virtually eliminates any other possible system for viruses

If however we would agree in principle that each good species of virus should have a suitable binomial and that we class related species in a single genus we immediately come up against the requirement which is the basis of modern systematics that the nomenclature should be based on a classification with a natural *i e* evolutionary significance The crux of the matter is to decide what is the natural criterion for deciding which of all the clones of virus that have been studied are sufficiently alike to justify saying that they are all examples of one species

Sometimes this is easy I do not think there would be any controversy as to whether or not mumps and herpes simplex viruses represent good species Each is responsible for a characteristic human disease that has been known in essentially its present form for centuries When any strain of either virus is compared with others from different parts of the world the differences among them are trivial compared with their common differences from any other known type of virus

The modern developments in population genetics which in the hands of Sewall Wright Dobzhansky and Mayr have led to a much clearer understanding of speciation in higher organisms are not directly applicable to agamic forms Work on recombination of viruses will have to go very much further before we can even consider that the concept of access to a common gene pool

can ever be added to the current picture of virus reproduction as wholly clonal in character

Nevertheless even with due regard to the highly mutable character of viruses the essential parasitism of viruses provides a very effective method of functional isolation and stabilization of the genotype Perhaps an approach is possible if we compare the situation not so much with the differentiation of higher organisms into *species*, but their higher level differentiation into genera families *etc* Systematists agree that the species is a more precise natural unit than the genus or any higher category yet to an outsider there still seems to be a considerable subjective element in the decision as to what is a species and what is a subspecies in certain groups Similarly, one cannot escape the impression that the higher categories do correspond to a large extent to natural situations and are not wholly creations for the convenience of systematists

In the most general terms we might follow Sewall Wright s conception of adaptive peaks in the field of possible gene combinations into the situation with parasitic microorganisms The virus of herpes simplex is the phenotype of a certain combination of genetic units which makes it uniquely fitted to survive in a given environment (which in this case is not only the human species but also special anatomical physiological and behavioral aspects of that species) It occupies an adaptive peak a peak which from the uniformity of natural strains of the virus is a sharply pointed one There is however, another environment in which the virus can survive indefinitely under conditions not much more specialized than the natural ones This is a 'well adapted experimental strain on the chorioallantois of the chick embryo Here there is not only the new tissue but also the requirements of the experimenter as part of the environment The required qualities are not usually present in the natural virus and in the first passages on the chorioallantois the genotype is in an adaptive valley The genotypic change is gradual and although the process has not been analyzed in detail everything points to its being essentially similar to that worked out by Demerec for the adaptation of the staphylococcus to a penicillin containing medium Random mutations occur in all directions occasionally toward a state better able to cope with the new environment Such favorable mutants prosper at the expense of the original and one eventually becomes the dominant form From this further mutants can arise which in their turn are still better fitted to multiply freely in what was initially an abnormal environment Eventually the species can be regarded as occupying a new adaptive peak

In higher organisms reproducing sexually, the diversity of forms evolving from a single ancestral gene pool (species) may be due to the occurrence of gene mutations or of chromosomal modifications *e g* polyploidy and the recombinations of the various genetic characteristics that exist within the Mendelian populations existing at any particular period In microorganisms such as viruses and bacteria mutation is the only important process so far demonstrably responsible for the development of new characteristics Recombinations of qualities can occur under laboratory conditions but it has yet to be shown that this is of any evolutionary importance For all practical purposes

we have to deal with clonal evolution, yet the end result is strikingly similar to that observed in the sexual evolution of higher animals and plants There is the same diversity of creatures and it is not a continuous diversity but a discontinuous one There is also the same strong indication of a hierarchical system of discontinuity We must assume that the two types of evolution can lead to very much the same end result

Probably the outstanding feature of the evolutionary process in parasitic microorganisms is the unimportance of the individual A few influenza virus particles initiate infection in one individual of a susceptible human community and an epidemic of some thousands of cases results From the point of view of the virus we have a series of precipitate population increases followed by catastrophic destruction In each individual infected the peak population of virus particles probably exceeds 10^{12} but it is certainly rare for even 10 of these to find opportunity for continued multiplication When an active epidemic is in progress over a populous area we might conceivably have 10^{17} virus particles in a viable state A few weeks later there may be no viable particles whatever in this particular environment

The evolution of sex may be regarded as a means of retaining and recombining elements of mutational novelty in a more economical fashion than is possible with nonsexual organisms Where numbers of individuals are virtually unlimited mutation rate high and generation time a few hours at most there is no need for economy to conserve the useful mutation

Two examples of an evolutionary process in viruses have come sufficiently within my own field of work to justify a little further discussion of the process of speciation The first is in regard to the St Louis Japanese B, and West Nile group of encephalitic viruses to which we have recently added Murray Valley In our hand Murray Valley and Japanese B have only an incomplete immunological relationship of much the same character as between St Louis Japanese B and West Nile and I understand that Dr Smadel s group are in general agreement

Although probably too few strains have been studied from the various regions concerned to allow us to be dogmatic it seems to me that in this field we may have something equivalent to the process of geographic speciation that occurs in higher forms The endemic regions at present seem to be discontinuous but there are some extremely interesting points about the Japanese B-Murray Valley relationship Japanese B antibodies have been observed in Guam and Murray Valley antibodies in North Queensland so that there is a strong suspicion that viruses of the group may extend continuously along the margin of the western Pacific A detailed serological study of the viruses from selected points along that arc might throw a lot of light on the process of virus evolution I should consider that with these viruses serological character is a nonadaptive feature the change being accidentally associated with adaptive modifications for survival in the particular ecological complex—birds mosquitoes and climate—characteristic of the different regions

What might be called temporal evolution may be seen in the changing character of influenza A The stock strains SW15 WS PR8 (o MEL) and

FM1 (or CAM) have antigenic qualities that allow very easy separation by anti H A tests Were it not for the common complement fixation antigen, they might well be considered a distinct species

Work done by Anderson in my laboratory on the hemagglutinin inhibition capacity of human sera from different epidemics showed much variation from serum to serum but the general picture emerged that the majority of broad human sera contained antibody against infecting strain and against A strains that had been isolated in past years but very little against 'future strains We have put forward the hypothesis that influenza A virus survives in our current civilization of extensive and active movement all over the globe by a process of continually emerging serological novelty Obviously influenza has to move always through a partially immune population and serological novelty would be an advantage to survival except in isolated communities away from the main masses of population The general experience of workers with influenza A is that there has been a continuing series of changes in serological character, WS, PR8 and FM1 representing convenient examples picked out from a changing continuum Each new serological type seems to replace the preceding one very rapidly The first A strain CAM was isolated in Australia in 1946 The epidemics in the northern hemisphere in January 1947 were all of the new type It is in line with this hypothesis that Mulder found that CAM showed a more definite antigenic relationship to PR8 than did FM1

In this view the serological character of influenza A (and probably B) is a highly adaptive feature that introduces a type of evolutionary change that has no clear analogies in higher forms although perhaps a military historian might find parallels in the evolution of military weapons and tactics Perhaps a real analogy is to be found in the history of the wheat breeder s struggle to produce strains resistant to rust New physiological strains of the fungus always seem to arise to plague him

All that I want to underline is that an evolutionary interpretation of the existence of a discontinuous range of virus forms is possible along lines that are based on the classical discussions of speciation in higher forms It may be many years before a complete interpretation of the evolutionary development of the viruses is developed and accepted I would not exclude the possibility that it may become necessary to consider some viruses as representing completely different orders of being from others but for the time being and for the purpose of nomenclature and classification it is certainly expedient that we regard them as living organisms I am not impressed with the contention that classification must wait indefinitely for even a beginning to be made The fundamental techniques of virology are now soundly established and are sufficient to provide the solution at least in principle of the practical medical and economic problems raised by the existence of virus disease I do not foresee any great spontaneous activity in fields of virology that will greatly advance the knowledge of systemic relationships Advance will continue as at present to be dominated by the need to develop methods of control of virus diseases of practical importance and by the study of the details of composition the processes of infection and multiplication in species specially favorable for labora

tory study Vaccinia and influenza viruses the T-even group of dysentery coli phages and tobacco mosaic virus are the current favorites It may be pessimistic so to believe but neither of those directions of study seems likely to do more than add occasional sidelights on the problem of classification and nomenclature

I think that a start on the work should be made now and that the function of this monograph should be to provide guidance as to how that first step should be made so that at least some progress can be consolidated before the next International Congress of Microbiology convenes

My own attitude would be in line with the decisions of the Rio Congress but might be put slightly differently Taking in the first instance the viruses whose hosts are warm blooded vertebrates the logical procedure is to look over the whole range of characters presented by the various clones of such viruses as have been studied and recorded Out of that diversity we can see certain groups of viruses that seem much more closely related to one another than to any viruses outside of their own groups Goodpasture nearly 0 years ago recognized this in regard to the pox viruses and his arguments have been given more force since then by the demonstration of the electron microscopic appearance of the virus particle

As a working rule it might be suggested that viruses falling in one genus have approximately similar size and appearance in electron micrographs and at least one common functional characteristic Species within the genus might be defined as containing all strains of similar serological structure

That working rule like any working rule in biological systematics will be subject to the individual opinions of workers interested in the group being considered Splitters and lumpers are as likely to appear amongst virologists as amongst ornithologists but this should not make broad agreement impossible

There are some points of special difficulty with viruses that need discussion I shall illustrate them only in terms of animal viruses responsible for human disease but similar difficulties will probably arise in the other major groups as well

The first is the mutability of viruses particularly in relation to the frequent necessity of modifying the virus before it can be subjected to laboratory study I have frequently pointed out that the wild strains of influenza A virus responsible for epidemic influenza will not multiply in the allantoic cavity will not agglutinate chicken cells and will not produce lung lesions in mice All these characteristics are those of strains adapted to growth in the convenient laboratory animal

In standard systematic work it is understood that when a new name is given one individual specimen is to be designated the type of the species and deposited in some suitable repository where it is available for subsequent study by other systematists In bacteriology the type culture collections fulfill this requirement I realize that there may be differences of opinion on the point but I should prefer to see definitive names attached only to well studied viruses of which a certain clone can be maintained as the type specimen of the species

The influenza viruses have been studied more closely than any other type of animal virus and, no doubt the WS strain of Smith, Andrewes and Laidlaw has much the strongest claim to be type strain of influenza A In my laboratory I have three strains all of which I am certain are descendants of the virus that caused WS s attack of influenza in January 1933 The properties of the three are however extraordinarily different One is neurotropic in mice and two are capable of producing hemorrhagic death in chick embryos The hemagglutinin of one is destroyed by heating to 52° for 30 minutes that of another has to be heated to 67 C All obviously differ greatly from the parent wild strain

A definition of the species influenza A virus might therefore require a statement something like the following Influenza A virus is the agent responsible for extensive epidemics of human influenza including that in Southern England in 1933 from which the parent form of the type strain WS now deposited in X collection of type viruses was isolated Strains of influenza A virus when isolated and adapted to allantoic passage have such and such characters in common with the type strain and produce a soluble complement fixation antigen reacting with suitable antisera in the same fashion as the antigen produced by type strain WS

Where a well defined human disease has all the characteristics of a viral infection but the virus cannot be studied in the laboratory, the question of giving the virus a name is perhaps a rather unreal one The only inconvenience that might be overcome is the difficulty of remembering what is the French or German for measles and German measles Outside the human field however there are animal diseases such as the poxlike diseases of sheep and goats, or the immense range of plant diseases that have not yet been subjected to full comparative study for which accepted but provisional names would be a convenience The suggestion that a group of provisional names for what have been termed Imperfectly Known Viruses should be adopted is a reasonable one provided machinery is available for the adoption of definitive names when relationships have been clarified

There is already in existence a published classification of rickettsiae animal plant and bacterial viruses in the last edition of Bergey I am certain that all virologists who have to deal with animal viruses agree that there are some serious misplacements of viruses in that classification which under any circumstances would have to be corrected On the other hand I am rather attracted to Holmes s use of the names of traitors and derogatory epithets from the classics as generic names It is going to be very difficult to find names with a direct indication of a distinguishing feature of a genus The other alternative of attaching to each genus the name of some investigator who made an outstanding contribution to the knowledge of the group concerned is probably that most in line with current bacteriological practice The difficulty of euphony tends to arise as in Holmes s *Miyagawanella* Goodpasture s *Borreliota* is acceptable and the possibility of replacing the conventional -ella termination by iota for viral genera compounded with proper names might be considered but one can easily imagine that some appallingly inconvenient words for

example *Landsteineriola* for the polio viruses would result, if the rule were followed The form of a name however matters little once it has been accepted and used I rovided it is reasonably euphonious and has some association with the history or character of the group anything will serve

May I in conclusion attempt to summarize what I think are the problems on which this monograph should concentrate? In the first place there is probably a danger that discussion of the classification of individual groups may turn too much on details that have no relevance to the general problem Might I ask all contributors to consider to some extent at least whether the characters of their particular group can provide leads toward an acceptable general approach?

The problems for consideration are as follows

(1) Is what may be called the Rio principle acceptable viz that for the time being only those groups of viruses that have been extensively studied should be regarded as ready for treatment or is it desirable that an internationally acceptable name should be attached to every type of virus that is distinct enough to require a name?

(2) If any Linnean or other nomenclature is adopted, the question of types for species and genus can hardly be avoided I have already given an example in the form of a tentative definition of influenza A virus to indicate what extensive difficulties there are in relation to the maintenance of type clones of a virus and their relation to the wild type I can see no escape from the designation of a type strain for each valid species and of a type species for each genus Others may feel that it mere pedantry to deny that measles virus for instance is a valid species simply because there are no laboratory strains in existence

(3) Can any agreement be achieved as to what are generic as against specific characters? To what extent must clones of virus differ to be accorded species rank? The old rule that the systematists should as far as possible disregard recent adaptive characters may be particularly sound in virology Host range and virulence are clearly of little systematic value Tissue preferences and capacity for transfer through an invertebrate host reactivity with cell surfaces as in influenza viruses and bacteriophages susceptibility to inactivation by physical and chemical agents serological character and finally size and electron microscopic morphology will all need assessment from this point of view

(4) Should monotypic genera be allowable? In the field of animal viruses rabies is the outstanding example

(5) Where there is a well-defined group *e g* the pox viruses should poorly studied or rather distant types—swine pox and rabbit myxomatosis in the present example—be provisionally included or kept outside until further study clarifies the position?

(6) How far is it expedient to go in regard to higher level classification of the viruses *i e* in categories higher than genus? The most interesting of all the problems of classification whether plant animal and bacterial viruses have a common evolutionary origin or whether even within any of the three great

groups of known viruses there are diverse evolutionary origins, is I think too remote from present-day knowledge to justify discussion in this monograph

May I conclude by suggesting that there are two important reasons why we should go all out to make a start on virus classification?

The first is that any classification will act as a stimulus to virologists to try to better it and in the process, to consider more deeply the evolutionary significance of differences between viruses (or any other groups of pathogenic microorganisms) I have held for a long time that such an approach is not only immensely absorbing as a mental exercise but also desirable for the practical understanding of such diseases as influenza and encephalitis

The second is that an accepted classification and nomenclature will make it easier for those outside our own group of professional virologists—students physicians plant pathologists and biologists generally—to understand our science

From the very nature of the evolutionary process any classification must be imperfect I think in the circumstances we should strive for agreement rather than perfection

In one final word I should apologize for building this opening paper almost wholly around the problems of the animal pathogenic viruses Nevertheless apart from questions of origins the same broad principles apply to all three groups, and I hope that what I have written is not wholly without application to plant and bacterial viruses as well

CONCEPTS OF CLASSIFICATION AND NOMENCLATURE IN HIGHER ORGANISMS AND MICROORGANISMS

By Ernst Mayr
The American Museum of Natural History New York N Y

It would be impossible to comprehend the unbelievably great diversity of nature without naming and classifying the units of which it is composed Classification of course is not something confined to animate nature We classify books in a library we classify rocks or gems laws and regulations types of weather in short any variable phenomena

All classification involves two steps The first one consists of the definition of units their description and the finding of diagnostic differences between these units in short analysis The second involves synthesis namely the assembling of the units into groups and their arrangement into an hierarchy of ever larger groups We must understand this process of classification if we are to arrive at a sound nomenclature

What is the basis of our current system of zoological nomenclature?

Systems of Nomenclature

Names are recognition symbols In biology they are an international language that makes the repetition of detailed descriptions unnecessary Names for organisms have existed long before scientific names were given Even the most primitive tribes in Africa South America or New Guinea have vernacular names for animals and plants They are usually uninomial expressing distinction such as skunk elk robin or flicker Beginnings of a binomial nomenclature consisting of a combination of a group name (generic name) with a specifying name (specific name) are also sometimes found not only among pre-Linnean authors but even among primitive people It was however Linneus who made it the basis of a consistent system of nomenclature that of binomial or trinominal nomenclature

Every animal or plant has a scientific name according to this system which consists of two words. The house sparrow for instance is *Passer domesticus* *Passer* is the generic and *domesticus* the specific epithet The precise significance of binomial nomenclature was not entirely clear to Linnaeus nor to most of his successors In fact it is not clearly understood by many of our contemporaries The two names of which a scientific name is composed have actually opposite functions The specific name expresses distinctness the generic name relationship It is evident then that binominal nomenclature in order to be meaningful must be based on sound classification Sound classification in turn is founded on a thorough knowledge of the units which are to be classified and improvements of classification are ultimately attainable only through improvement of our knowledge of these units

The history of all classification whether dealing with inanimate objects or with organisms shows that early attempts of classification are based on superficial similarities and very often on single characters while all improvements of

classification are due *to an ever more deeply penetrating* analysis and a broadening of the basis of classification by including more and more characters The soundest classifications are those built on the greatest possible number of clues Reciprocally it can be stated that, in sound classifications there is usually a fair concordance of the various characters The question of the reliability of taxonomic characters is an important one but too broad a subject to be pursued further in this connection I must refer to more extensive treatises

The problems of nomenclature that are confronting the virologist are whether or not the time has come to apply binominals to viruses and if so on what principles to base such nomenclature The knowledge of the higher animals was already far advanced when Linnaeus introduced the system of binomial nomenclature in zoology In 1758 when this was done nearly all of the more common species of mammals and birds of Europe had already been precisely defined It is well to remember this when attempting to apply the system of binomial nomenclature to such poorly known organisms as the viruses are

The second step the synthesis of the lower categories (the species) into higher categories (genera families and so forth) has lagged far behind the first one Many of the higher groupings are still very dubious Although the last North American species of birds was discovered in 1889 we are still in doubt as to how many genera of North American birds to recognize and are completely in the dark with respect to the delimitation and relationship of families We may conclude from this that the application of binomial nomenclature requires a fairly advanced knowledge of the basic units but is not too much interfered with even by fairly extensive ignorance of the arrangement of the higher categories

A precise knowledge of the units to be classified is essential but not enough *What is also needed in biological classification are classifying principles which* help the systematist to devise sound classifications

Two facts more than any others have led to the chief conceptual improvements of our system of biological classification The first is the principle of evolution It is now realized that the similarity of organisms and of groups of organisms is not a freak of nature but is due to the fact that similar species and genera are descended from common ancestors The natural system' of the taxonomist then reflects phylogeny In fact some authors have gone so far as defining taxonomy as the scientific classification of the different kinds of living organisms according to the proved or inferred phylogenetic relationships This concept is based on *the belief that organisms that have descended from* the same ancestor will have more characters in common with each other than with any other kinds of organisms It is now known that this is not always the case since selection pressures may lead to a considerable divergence from the ancestral type For instance birds and crocodiles are phylogenetically closer to each other than are crocodiles and turtles or crocodiles and lizards Yet the conquest of air by *Proavis* has led to such a dramatic reconstruction of the avian system that birds are now much more different from crocodiles than the latter are from other reptiles

The theory of evolution has had its main effect on the *synthetic* processes of classification the definition of groups and their arrangement in an hierarchy of categories A different biological concept has greatly influenced the results of the *analytical* stage of classification namely the definition of the units of the taxonomist which he calls species I am referring to the concept that the process of sexuality leads to a genetic integration of natural populations into species

Modern studies by systematists ecologists and population geneticists have made it abundantly clear that the species is a unit as important and meaningful in biology as the cell or as is the atom in physics In fact the species occupies a central position in the hierarchy of organisms In order to understand the true significance of species it is necessary to say a few word about the biological meaning of sexuality The significance of sexuality is genetical Sexuality permits recombination of gene complexes and thereby provides an inexhaustible store of genetic variability Modern researches have shown conclusively that the most objective property of species is perhaps the gap between different species It is the place where gene exchange is interrupted Species then can be defined as populations that are separated from each other by a reproductive gap We shall return presently to the definition of species

Sexual reproduction is *par excellence* characteristic of higher organi ms particularly of the higher animals They nearly always live in a generalized variable environment with a great complexity of external conditions High genetic variability is a great advantage under such conditions Although the more extreme variants will be eliminated by the environment in each generation the total variability will be continuously restored by recombination as a result of the sexual process The greater the variability the greater the opportunity to utilize the variability of the environment

It appears that condition may arise in nature which may place a selective premium on the temporary abandonment of sexual reproduction One such situation is when an empty niche must be filled rapidly and selection pressure is very much reduced This is true for instance for fresh water plankton which arrives in previously vacant lakes or for plant lice which in spring have suddenly available an inexhaustible food supply on freshly developing leaves The production of parthenogenetic females each of which again produces only females will lead to a much more rapid filling up of the niche than the production of 50 per cent females and 50 per cent males

In view of the importance of interbreeding populations for the definition of species it is evident that information on the presence or absence of sexuality is of vital importance to the taxonomist It is precisely with respect to *this* subject however that information is still very deficient on viruses What little information there is suggests that sexual processes may be widespread if not univer al among viruses but that there i often a temporary abandonment of sexual reproduction

Genetic recombination, that is, sexual reproduction, has been recorded up to now in only a few viruses In the cases in which it was seriously looked for however it was nearly always found The assumption of the widespread occurrence of sexuality in viruses is backed also by the following consideration

Virus strains fall normally into well-defined groups Influenza A mumps *etc* If reproduction was strictly asexual one would expect mutation in the independent strains to have such a pronounced centrifugal effect as to obliterate eventually all traces of groups The existence of groups indicates that occasional gene exchange between diverging conspecific strains prevents the dissolution of the groups The frequency of recombination may be very low The chance that it will be discovered if it occurs in one of 5000 generations is very small Furthermore the occurrence of sexuality may depend on very specific environmental conditions as in parthenogenetic higher animals or on multiple simultaneous infections

In view of the scarcity of available information however, it would be premature to study the ecology of this potential "alternation of generations" as we may call it There are various factors that might place a selective premium on temporary asexuality If a new host is invaded it is highly advantageous for the virus to reproduce at maximum speed, before immunity reactions develop This would favor temporary asexuality Additional factors however may play a role Viruses being adapted to cells of a single host live in a much more uniform environment than free living higher organisms Great genetic variability might be actually a disadvantage with them As soon as a superior that is highly viable gene combination is found it will be advantageous to reproduce this superior gene combination asexually rather than to try to improve it by genetic recombination at the risk of destroying it It is very probably that microorganisms have a much less complex genetic system than higher organisms that the gene complexes are less well "buffered" and that every mutation affects the phenotype more conspicuously than in higher organisms If so there would be a premium in such a system on the reduction of sexual reproduction and on a rise in the rates of growth and reproduction

It is possible that in such a system mutability may in part take over the function of recombination in higher organisms Considering the enormous population size in microorganisms and the high number of generations per time unit even a low mutation rate can provide an amount of variability that might offer all the needed material for selection in a slowly changing environment

For all these reasons it is evident why there is so much (temporary) abandonment of sexual reproduction among microorganisms The classifying taxonomist then will have to deal both with sexual populations and with asexual strains or lines We must keep this fact clearly in mind when we try to place microorganisms into the categories which have been defined for sexually reproducing higher organisms

The Meaning of Categories

The taxonomist classifies his material by arranging it into categories As stated above the species is the basic of these categories, and it is therefore necessary to say a little more about the meaning of the category *species* The species concept goes back to Plato to whom species meant kind This concept still lives outside of biology as for instance when a mineralogist speaks of species of minerals From this original species concept arose that of the

naturalist which was for the first time clearly expressed by the British botan st Ray about 1690 This is the concept that was taken over and formalized by the Swedish naturalist Linnaeus It is based on a study of local faunas and floras in which each species is usually clearly separated by a definite gap from every other specie Such a clear definition and separation of species occurs only in a local situation at a single time and this species concept has therefore also been referred to as the *nondimensional species* What is more important this species concept is based on the observation of interbreeding *within* the population that belongs to a species as against the lack of interbreeding *between* populations belonging to different species In other words it is a concept strictly applicable only to sexually reproducing organisms The modern bi ological species definition may be stated as follows *Species are groups of inter breeding natural populations that are reproductively isola ed from o her such groups* This biological species definition is the end product of a long conceptual evolution It is a far cry from the morphological and typological species concept of Linnaeus and the early taxonomists

The Asexual Sp cies Since interbreeding is the *ultimate test of conspecifi city* in the higher animals and since this criterion is unavailable in asexually reproducing organisms it is evident that the species concept is difficult to apply to such forms How should the taxonomist treat clones pure lines biotypes and so-called strains or stocks? The belief is growing that most if not all forms of asexual reproduction are not in the least primitive but a derived condition All such asexually reproducing lines will terminate sooner or later either by extinction or by fusion with another line through a sexual process There is much evi lence that many of these lines are started by single mutations To admit such lines as full species of equal standing with the species of higher organisms would be very confusing The solution that appeals to me most is to consider all asexually reproducing descendants of a single sexual species as a collective specie This would include all strains that owe their origin to mutation with the exception of polyploids In practice it may be rather difficult to establish the relationship of such strains and it may be necessary to adopt a provisional solution until the final relationship is established Since in such asexual strains many of the so called species criteria are subject to mutation the final decision will have to be based on an evaluation of the multiplicity of characters among which the serological ones are to be given special weight

The binomial nomenclature has a well-defined significance in biology and it is not permissible to give binomials to biological entities that do not deserve them It is advisable to employ a vernacular nomenclature combined with symbols and numerals to designate all situations that are not fully understood The geneticists have proven that it is possible to name tocks and strains accurately without recourse to binomials

The *subspecies* is the only category below species level recognized by the taxonomists It has a very definite meaning in systematics A subspecies in a sexual species consists of groups of interbreeding local populations that are taxonomically di tinct from other such groups The differences between sub-

species are usually due to local selective factors not completely compensated by gene flow Subspecies in free living animals are, therefore characteristically geographically definable In parasites, an interruption of gene flow is often caused by host segregation The subspecies of parasites are therefore, normally host races The subspecies concept is closely connected with the population concept of the sexual species No one has as yet succeeded in establishing subspecies in asexual species

The *variety* is a concept linked with the morphological typological species concept of Linnaeus Any deviation from the species type was described as variety, no distinction being made between individual variations and local races Since it has become customary to apply the term "subspecies' to local races, the term ' variety is used by zoologists more and more for individual variants within populations A trend in the same direction is recognizable in botany, although many plant taxonomists still use the term variety interchangeably for geographical races or for individual variants It is possible that the term variety' would be suitable for a designation of strains and clones within asexual species

The *genus* is a group category It comprises a group of similar or closely related species In view of the fact that species are not evenly distinct from each other, but are often found in clusters of closely related ones more or less distinct from another cluster of related ones the genus is based on an objective phenomenon of nature There is no objective criterion of generic difference available however comparable with the criterion of noninterbreeding in the case of species The delimitation of genera is therefore subjective While it is the function of the species to denote distinction it is the function of the genus to denote association As interpreted by the biologist the genus denotes relationship The generic name has as its function the relief of the memory by grouping the almost infinite multitude of specific names The genus has been defined as follows *A genus is a systematic category including one species or a group of species of presumably common phylogenetic origin separated by a decided gap from other similar groups* The history of the taxonomy of the higher organisms shows that it is advantageous at the beginning to have large genera If it turns out that they are composed of highly heterogeneous elements they may subsequently be split

The greatest practical difficulty in virology appears to be that of ranking the recognized units and groups When units are to be grouped an author will have to determine carefully whether he is dealing with strains that are members of a collective species or whether he is dealing with species that are members of a genus The choice between these two alternatives is likely to be the most difficult problem in virus taxonomy

Rules of Nomenclature

Finally a word about rules of nomenclature Perhaps the virologists can learn from the experiences of the zoologists Three points have become very clear to me One is that every name whether of a lower or of a higher category, must be clearly based on an objective type Methods of investigation

are continuou ly changing and unless objective types are deposited it is impos ible to re examine at a later date the unit on which a name is based

The second point is that it would be most advisable to shift the responsibility for names from individuals to either national or international committees If this is not done there is the danger that a most unscientific race will develop by authors, all over the world trying to get credit for new names Let there be an official list of scientific names of viruses and let the author apply to the committee to have certain names placed on this list the committee being o to peak, the author of each name Let there also be a rule that no scientific name can be used in a publication unless it is on this official list In zoology such a procedure unfortunately is not possible in view of the millions of existing species Such a procedure might however be feasible in virology

Finally having an official list will eliminate the greatest stumbling block of stability in nomenclature namely the rule of priority The never-ending turmoil in zoological nomenclature produced by this unfortunate principle can be eliminated if an official list of valid names ba ed on well-defined units is made the final arbiter of all names This would help to attain tability the ultimate object of all nomenclature

VIRUSES THAT REPRODUCE IN PLANTS AND INSECTS*

By L M Black†

Brooklyn Botanic Garden Brooklyn N Y

There exists a group of viruses that probably links some plant and animal viruses very closely together The members of this group multiply both in their animal vectors (insects called leafhoppers) and in the plants they infect Their existence forecasts a time when the current primary divisions of the virus world into those attacking bacteria plants or animals may have to be revised or abandoned in favor of some other basic classification It is not the purpose of this paper to propose any such drastic action at this time The primary division of viruses into bacterial plant, and animal groups is useful at present and is likely to continue to be so for some time to come

It seems appropriate in this monograph, however to review the evidence on this topic because it has an important bearing on our outlook on virus relationships because it has been a controversial subject for several years and because the evidence is scattered in a variety of publications

In the discussions of experiments bearing on the problem of virus multiplication in both plants and animals only considerations that seem to me to have a critical bearing on the question will be dealt with These will be discussed whether or not they have been raised before On the other hand some considerations that have been advanced but that do not seem pertinent to the main question will be treated very briefly or omitted

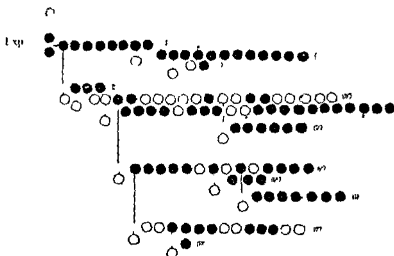
There are four viruses (rice stunt virus *Fractilinea oryzae* (Holmes)[17] aster yellows virus *Chlorogenus callistephi* Holmes clover-club-leaf virus *Aureogenus clarifolium* Black and wound tumor virus *Aureogenus magnivena* Black) for which evidence of their increase in hosts belonging to the plant and animal kingdoms has been obtained All four are viruses transmitted from plant to plant by leafhoppers in which they undergo a long incubation period (at least ten days for rice stunt virus[16] at least nine days for aster yellows virus[22] about three weeks for clover club leaf virus[7] and at least 13–15 days for wound tumor virus[4]) In all of these the ability of the vector to transmit the virus persists for long periods often for life after the incubation period is completed without the vector having any further access to virus from plants Oman[26] listed 22 plant viruses transmitted by leafhoppers and more are known today Probably many of them have the ability to grow in both their plant hosts and insect carriers

Experiments with Rice Stunt Virus The first evidence of this sort was adduced from studies on the virus causing rice stunt in Japan This virus was the first plant virus demonstrated[13] to pass through the egg of its insect vector to the progeny Using the vector *Nephotettix apicalis* Motsch var *cincticeps* Uhl Fukushi[15, 16] demonstrated that the virus from a single infective female could be passed through the egg to six succeeding generations without replen

* Th i st g t f th tho eferred t i th s publ c t o w s pported, I p rt by a gra t from the Am c Can Soc ty w d d ommend t o f th Comm tt o G owth

† P s t ddre D p rtm t f Bot U v rsity f Ili U b Ill

ishment from plants FIGURE 1 shows Fukushi s diagram of the descent of infective and noninfective progeny from the single infective female u ed to begin his most significant experiment Only one infective male the first was used in this experiment and Fukushi had already shown that the virus did not pass to the progeny from the male parent He insured that the insects in the experiment obtained no virus from plants by removing each individual to a fresh healthy rice plant *Oryza sativa L* at the moment of its hatching from the egg Fukushi[13] states that no instances were observed in which viruliferous insects emerged from eggs deposited in infected plants by virus-free females



● ○ indicate infective and non infective leafhoppers, respectively

FIG 1 Diagram how g th descent of inf t and noninfect le fhoppers Fukush xperiment 5 (Aft Fukush)

The newly hatched nymph was usually kept on the first plant for five days and then transferred to a fresh healthy young rice plant on each succeeding day until it died None of the insects in the experiment infected plants earlier than nine days after hatching Therefore no virus could have been introduced into and recovered from the first plant on which each insect fed for five days It is most improbable that virus could have been obtained from any of the *subsequent plants supporting the insects for a single day for the following* reasons Fukushi s[14] data indicate a minimum incubation period in plants of six days (most incubation periods were between nine and 13 days) Since it is the general experience that insects can recover virus from plants only a day or so before the appearance of symptoms it seems unlikely that Fukushi s insects could have introduced virus into a healthy plant and recovered virus from it in a single day even though Storey[7] showed that maize streak virus *Fractilinea*

maidis (Holmes) McKinney could be recovered, in rare instances by non viruliferous insects feeding on the same leaf as infective insects during periods of one or two days The vector, moreover, does not seem to acquire the virus readily from rice plants [14]

Fukushi s experiment lasted for 374 days and during this time 82 infective leafhoppers, in six generations derived from the original female infected about 1 200 rice plants Fukushi considered that his findings demonstrated multiplication of the virus of rice stunt in its insect vector Bawden[1] considered it improbable that the virus multiplied, and he advanced many arguments against this interpretation Fukushi[16] answered these in detail Bawden[2, 3] reiterated his position It is not appropriate to enter into a detailed discussion of this controversy here Much of it is concerned with evidence that cannot determine the main point at issue namely whether the amount of virus in the female leafhopper with which Fukushi began his classical experiment No 5 was sufficient to produce all the infections in subsequent generations without multiplication in the leafhopper In this connection, Bawden[3] correctly points out that a single leafhopper egg might easily contain enough virus to produce infection in the more than 1 000 rice plants mentioned by Fukushi

A study of Fukushi s data however is more impressive than indicated by the total number of infections produced The total number of infected plants could have been greatly increased if the feeding periods had been shorter than a day as shown by data in his table 7[14] and table 30 [16] which demonstrate transmission in successive periods of less than one day These considerations however are not as important as the minimum dilution of the starting quantity of virus attained in his experiment Fukushi did not estimate this but calculations based on the information available in his papers are summarized in TABLE 1 They show that the virus present in the leafhopper with which he started his experiment No 5 underwent an estimated minimum dilution of 1 563 000 It should be remembered that this is a minimum dilution figure Several factors certainly make the actual value considerably higher The systematic errors mentioned below as reducing the calculated minimum dilution of the clover club leaf virus in a similar experiment apply equally well here The most important underestimation however results because Fukushi only reported the numbers of progeny he picked from plants at time of hatching and these numbers are much lower than the number of eggs one would expect a single female to lay It is quite likely that the actual dilution attained by Fukushi in the sixth generation is of the same order of magnitude as that obtained at the same point in a similar experiment on clover club leaf virus (see below) The dilution in the sixth generation in that case was at least 10^{-8}

If one assumes values estimated to give a minimum number of virus particles likely to be found in a leafhopper or its egg one obtains estimates of the order of 10^6 particles for the leafhopper and 10^4 particles for the egg These estimates are based on the assumption that the egg weighs as little as 1/100 of a leaf hopper weighing one mg, that the insect tissues contain as little as one part virus per million parts of tissue by weight (*cf* with proportions of plant viruses

in plant extracts bibliographical reference 3 table 14) and that the virus is as larg as the largest known plant virus (potato yellow-dwarf virus *Aureogenus vastans* (Holmes) Black) with a particle weighing approximately 490 × 10⁶ molecular weight units[11] Assuming values estimated to give a maximum number of virus particles likely to be found in a leafhopper or its egg one obtains estimates of the order of 10¹² for the leafhopper and 10¹¹ for the egg These estimates are based on the assumption that the egg weighs as much as 1/10th of a leafhopper weighing one mg that it contains as much as one part virus per 100 parts of insect ti sue by weight and that the virus has a particle with weight equivalent to a molecular weight as low as 6 000 000

As the dilution attained in Fukushi s experiment was probably at lea t 10^{-5} then assuming the minimum figures for the number of virus particles at the start of his experiment the ability of the in cts to infect plants would have shown a progressive decline Assuming on the other hand maximum numbers of virus particles it is uncertain whether progressive decline in infective ability

TABLE 1

MINIMUM DILUTION OF RICE STUNT VIRUS DURING PASSAGE THROUGH THE EGG OF ITS INSECT VECTOR FOR SIX GENERATIONS

C tion†	Numbe f p og y	I f t ty test ‡	Re ip oc l f d t on	
			E h ge t	T tal
1	10	8/8	10	10
2	6	3/3	6	60
3	45	7/19	17	1 0 0
4	30	11/14	24	24 480
5	42	7/13	23	563 040
6	Several	1/1	?	563 040

Calcul t d from d ta f T Fukush
† F k h ted th p ent gen rat on t b t her th first g ratio f prog y l ou t d as t
‡ Al in l d as insf tero thos indi i uals rev aled to b so only by progeny te ts

would have appeared Fukushi has very good information on th infectivity of the insects since he transferred each one to a fresh test plant each day as long as it lived His table 31 shows conclusively[16] that the percentage of plants infected showed no progre ive decline with dilution of the original virus nor did the percentage of infective leafhoppers in successive generations It should be pointed out that although Fukushi did not clearly indicate why the experiment ended with the sixth generation it is clear that it did not end because his leafhoppers became nonviruliferous

Experiments with Clover-Club-Leaf Virus When the clover-club leaf virus was discovered and found to pass through the egg of its vector *Agalliopsis novella* (Say) to a high proportion of the progeny[7] an experiment like Fukushi s was carried out[8] In planning the experiment with clover-club-leaf virus an effort was made to profit by the earlier work of Fukushi In particular an attempt was made to determine the total number of progeny from each female and the proportion that was infective No effort was expended in trying to see how many plants could be infected by any particular insect since the critical information desired was whether or not the insect wa infective From

the start, precautions were taken against the accidental termination of the experiment Reserve colonies were maintained in each generation and the virus-bearing females were outbred to virus free males from a stock colony to prevent debility due to inbreeding

The experiment was begun when a virus-bearing female leafhopper weighing 1 7 mg was mated with a virus-free male, and the pair was caged on a Grimm alfalfa plant *Medicago sativa* L As will be shown later, Grimm alfalfa is immune to the clover-club leaf virus The alfalfa plant was grown in soil that had been steamed to kill weed seeds The female produced 4? nymphs of which 21 were tested individually on a series of four crimson-clover plants, *Trifolium incarnatum* L and then discarded Fifteen of the 21 produced infections On the average therefore the virus in the original female had been diluted approximately 1 30 in each of her progeny The 21 remaining progeny were each placed on an alfalfa seedling and when they became adults, the females were mated to virus free males The virus has a long incubation period in crimson clover and in each generation insects had to be mated before any infectivity data were obtained on their sibs In this and subsequent generations, the pair that produced the greatest number of progeny was chosen to continue the main line of descent This was done in order to have a basis for selection that was independent of virus concentration and also to secure the greatest dilution possible Usually, about 30 pairs of insects were mated in each generation

From these additional pairs four supplementary families with more progeny than others and close to the main line usually were held in reserve These supplementary families were discarded as soon as it was known that virus was present in the main line of descent In each generation a sample of about 15 progeny from each of these five families ordinarily was tested individually on crimson clover to determine the fraction that was infective With some exceptions, each of these insects was fed on each of three plants for three weeks Another sample of about ten nymphs from each of the same five families was distributed one nymph to each of a number of seedling alfalfa plants to provide females for continuing the line

The experiment was continued for more than five years through twenty one generations of insects grown only on immune alfalfa without loss of infectivity TABLE 2 presents the data for only the main line of descent The dilution of the original virus assuming no multiplication in the insects exceeded $1 \; 2\,8 \times 10^{8}$

This dilution is a most conservative estimate because it does not allow for systematic errors underestimating the dilution of the virus For example it does not allow for virus remaining in the mothers, for deaths among the progeny before they were counted or for the fact that other data clearly showed that the tests of the progeny did not reveal all those that were carrying virus As an illustration if the infective fractions as revealed by the tests were real, the families with the largest number of progeny in each generation should have been free of virus many more times than they were Also several females from a family were sometimes saved and produced progeny that were tested Usually

all produced infective progeny. A case in point is the main line family in generation 19. Only one of ten insects tested was infective (TABLE 2) but five sister insects which had been saved for progeny produced infective offspring.

These experiments were controlled in several ways. The clover plants on which samples of insects from the main line were tested were matched in each generation with at least as many crimson-clover control plants on which either no insects or virus free insects had fed. None of these plants became diseased.

TABLE 2

CALCULATED MINIMUM DILUTION OF CLOVER CLUB LEAF VIRUS DURING PASSAGE THROUGH THE EGG OF ITS INSECT VECTOR FOR 21 GENERATIONS

Generation	Number of progeny	Infectivity test	Reciprocal of dilution	
			Each generation	Total
1	42	15/21	30	30
2	101	6/15	40	12 × 10
3	35	3/15	7	84 × 10^{2}
4	54	9/15	32	2 688 × 10^{2}
5	60	3/15	12	32 256 × 10^{2}
6	89	9/15	53	1 709 568 × 10^{2}
7	104	6/15	4	71 801 856 × 10^{2}
8	106	15/20	80	574 414 848 × 10^{4}
9	64	3/20	10	574 414 848 × 10^{4}
10	173	5/11	79	453 787 729 × 10^{6}
11	88	6/21	25	113 446 932 × 10^{6}
12	31	2/11	6	680 681 594 × 10^{6}
13	52	0/15	0(1)†	680 681 594 × 10^{6}
14	59	1/10	6	408 408 956 × 10
15	129	1/5	26	106 186 328 × 10^{11}
16	60	11/20	33	350 414 885 × 10[illegible]
17	114	2/15	15	525 672 327 × 10[illegible]
18	50	2/15	7	367 935 629 × 10
19	107	1/10	11	404 729 192 × 10
20	128	5/10	64	259 076 633 × 10[illegible]
21	105	1/10	11	284 929 351 × 10 or 2 8 × 10^{26}

A t Black

† Th w th only po t wh th bs f ru th f m ly w th th largest umbe of progeny es tated shift g t ma h f d ce t t th fam ly w th th ec d la g t mber f p geny It an m ly th th est f th latt f m ly happened t be g t and th t of he p ogeny f th b doned f m ly (cury) happe d t b p th g I p te of th g t t t t th po t t pe m bl mul ply th rec procal f he d l tio by 1 in tead f by 0 bec th oc rr f inf t de d ts m a h t lea t on f th prog y in th fam ly w infect

Since none of the pots in which the caged alfalfa plants were grown ever produced weeds, the alfalfa was the only source of food for the insects. After these plants had been freed of leafhoppers they were tested for the virus by means of insects from one of two colonies. The leafhoppers in these colonies had been obtained virus free, in one case by heat treatment and in the other by selection from insects collected in the field. To date virus-free insects after feeding on the alfalfa plants which provided the only source of food for the main line of descent during the five years have failed to infect any of 830 clover plants, whereas comparable insects after feeding on club leaf clover have infected 169 of 60? test plants. During the time of these experiments there

had been no spontaneous appearance of clover-club leaf virus in either of the two stocks of nonviruliferous insects

It was concluded that dilution of the virus present in the original female to at least 1 2 8 × 10^{26} without loss of infectivity, must mean that the virus multiplied in the insect vector during its five year stay on immune plants It has already been pointed out that 10^{12} virus particles approximate the maximum number likely to occur in a leafhopper Even a mass of hydrogen weighing the same as the original female (1 7 mg), contains only about 5 1 × 10^{20} molecules

Evidence, which Bawden[3] considered in his book (p 93) as being inadequate to demonstrate multiplication of this virus in its vector, was not presented[7] as evidence of such multiplication, but only as evidence for transmission of the virus through the insect egg On the basis of the evidence for transovarial passage of the virus through many generations, Bawden[3] inserted an addendum to this section of his book (p 104), stating that the clover-club-leaf virus seemed capable of maintaining itself indefinitely in its insect vector

Experiments with Aster Yellows Virus Three entirely different types of experiments have produced evidence that aster yellows virus multiplies in one of its vector insects

Loss of Infectivity in Heat Treated Insects Kunkel[19] showed that infective aster leafhoppers *Macrosteles divisus* (Uhl) after being kept at approximately 32 C for varying numbers of days were unable to transmit the virus when returned to temperatures of about 24 C Eventually however they usually regained their infectivity at the lower temperature without fresh access to virus The period of their inability to transmit was roughly proportional to the length of time they were held at high temperatures unless they were held at the high temperatures for 12 days or longer in which case they lost the ability permanently TABLE 3 compiled from Kunkel s data [19, 21] illustrates these conclusions convincingly despite some irregularity in the results This irregularity exists chiefly in comparisons of data derived from different experiments carried out at different times and may very well reflect variations in the starting concentration of virus or in some other condition that varied from experiment to experiment Comparisons of data within experiments reveal a consistent pattern

Kunkel also showed that if insects were heat treated while undergoing natural incubation periods the effect was greater than if the insects were already infective Kunkel interpreted these results as meaning (1) that heat treatments of 12 days or more destroyed the virus in the insects completely, (2) that heat treatments for shorter periods destroyed virus in proportion to the temperature and duration of treatment and (3) that the time required for regaining infectivity represented an induced incubation period in which the virus once more multiplied to an infective concentration The greater effect of the treatment on insects in which virus was still undergoing incubation was attributed to a lower virus content in these insects than in infective ones

In a number of ways Kunkel strengthened his interpretation and obtained evidence against the hypothesis that the heat treatments were affecting the

insects rather than the virus He showed that the virus could be inactivated in periwinkle plants *Vinca rosea* L by treatment in a hot room at 38–42 C for two weeks or by immersion of plants in a water bath at 40–45 C for a few hours The leafhoppers could be grown through their complete life cycle at 35 C in what appeared to be a normal manner[20] Furthermore leafhoppers freed of virus by heat treatment were readily rendered infective again by feeding on diseased plants

Bawden[1 2 3] considered that there were so many possible alternative explanations of these results that it would seem extremely premature to regard them as evidence for the multiplication of the virus in the vector In view of Kunkel s own work and other evidence presented below there would seem to be little doubt that Kunkel s interpretation is correct

Titration of Virus Content of Insects During the Incubation Period A second type of experiment[5] also indicated that aster yellows virus multiplies in its

TABLE 3

EFFECTS OF HEAT TREATMENTS ON ASTER YELLOWS VIRUS IN *Macrosteles divisus* (UHL.)

E pe im t	Temperat t tment	D y f heat treatment f llowed by d y el ps g b f f ct ty was egai ed
A1†	31	2–0‡
A2	31	13–00§
A3	31	7–3 10–3
A5	32	6–16 12-00
A6	32	1–0 2–7 3–8
A7	32	4–2 5–5 10–6
A8	32	7– 7–6 8–13 8-17
B†	36	1–1 2–3 3–3 4–6 5–8 6–13 7–23 8–00

Comp l d from dat f L O K k l n
† K kel repo ed xperim t labeled A in 193 B in 1941
‡ Th first fig d th umb f d y the i ects w kept t th temperat i dic ted in colum 2 th seco d figur dicates th da p t lapsed fte th heat treatm t before th in ect g ed th b l ty t t pl
§ Th ymbol 00 ind t th t th ins ts ev r g ined th b l ty t inf t pla t

insect vector A large virus-free colony of *Macrosteles divisus* was maintained on healthy aster plants *Callistephus chinensis* Nees for six days None of these aster plants became diseased The colony was then fed on plants affected with aster yellows for one day and subsequently transferred at frequent intervals to healthy aster plants The colony infected none of these plants for 16 days but infected each of two plants on which it fed on each of the following 14 days Counting the day on which the insects fed on diseased plants as the first day two samples of 50 insects each were withdrawn at random from the colony on the second fourth eighth twelfth and sixteenth days and tested for virus at different dilutions Uninoculated insects and insects inoculated with juice from insects which had been fed all their lives on asters with yellows were included as controls *

* In ord t test for rus th f llow g p oced was rried t t 0 C Th mpl f in ts was grou d Suffi t ral 0 85 pe en N Cl sol ti was dded t d l t th w igh d leafhoppers th d d amount Th uspe w cl d by low-speed ent f g t d min t q tit es f om a h d l tion w jected by gla p llaries in o 320 young vi us-fre d lts. These w th pla ed immun ry pl t d ma ta d th g enh f th ee weeks wh l th ru p sed through th incubat on pe od 3b s rv g i jected inse t w then test d in t lo of fi pla ts ach lo y f d on pl t for on w k d on econd plant f tw weeks.

The results reproduced in TABLE 4 indicate multiplication of the virus in the insects at least a hundredfold between the second and twelfth day of the incubation period It should be pointed out that during this time, the source insects were maintained on susceptible aster plants, none of which became diseased It was impossible therefore for the insects to have introduced virus into these plants and to have withdrawn virus from them, after its multiplication in the plant host

Since these results were published three additional experiments of the same sort have been carried out by the writer In some the insects which were titrated for virus increase during the incubation period were maintained on rye *Secale cereale* L which is immune to aster yellows virus Altogether seven experiments of this type were performed Only two of the experiments gave good results but in all of them the results were consistent with those in TABLE 4 in showing an increase of virus during the incubation period

TABLE 4

TRANSMISSION OF ASTER YELLOWS VIRUS BY COLONIES OF FIVE LEAFHOPPERS INOCULATED WITH VARIOUS DILUTIONS OF JUICE FROM INSECTS FED ON DISEASED ASTER PLANTS FOR ONE DAY

Day f inject †	R t o f numb f colon fect to umbe of l es tested				
	Dil ti f ju ce injected			C t l i ect	
	10^{-}	10^{-2}	10^{-3}	U ocul ted	I ocul ted‡
2	0/19	0/20	—	0/17	17/19
4	0 19	1/19	—	0/18	18/18
8	—	3/20	1/20	0/19	19/19
12	—	13/14	18/18	0/18	20/20
16	—	7/24§	9/19	0/17	17/1[illegible]

Aft Bl k
† Dur g thes d ys j f om r n ects w ai t d t to t t ts
‡ I ocul ted w th fect n t j ice d l t d 10^{-3}
§ In th as ly 24 e t surv ed and they w test d Indi id lly on ast rs

Bawden[2, 3] has claimed that in these experiments the number of infections obtained at higher dilutions is usually greater than that obtained at lower dilutions In spite of considerable variation the published results with aster yellows virus and many unpublished experiments by the writer show a decrease in number of infected insects with increasing dilution This objection probably would not have arisen if the writer had published the results of his experiments in greater detail For example the data on the twelfth-day titration in the above experiment published as reproduced in TABLE 4 indicated that more infective insects were obtained by inoculation with juice diluted to 10^{-3} than 10^{-2} The data on which the figures of TABLE 4 are based are presented in TABLE 5 Study of this table reveals that, in this particular case the best comparison between these dilution is to be found in the results on the first lot of test plants These results showed that, at dilution 10^{-2} 11 out of 14 colonies were infective whereas at dilution 10^{-3}, only 11 out of 18 colonies were infective although about twice as many insects, inoculated with dilution 10^{-3} survived the test period of one week as survived in the group inoculated with

dilution 10^{-2} The infections on the second lot of test plants (and consequently the combined data for both lots of test plants) are greatly weighted in favor of dilution 10^{-3} as compared with those of dilution 10^{-2} because of the much better survival of insects injected with the former dilution It frequently happened in these experiments that the mortality was higher when insects were injected with the more concentrated suspensions of insect juices Actually therefore the one apparent exception in TABLE 4 to a decrease in infective

TABLE 5
EXPERIMENT A3D 1940 COMPLETE DATA ON TITRATION OF ASTER YELLOWS VIRUS ON 12TH DAY OF INCUBATION OF INSECTS

Colony	Dilution 10^{-3}					Dilution 10^{-2}				
	Ins.† 4–13	Inf.	Ins.† 4–20	Inf.	Ins.‡ 5–2	Ins.† 4–13	Inf.	Ins.† 4–20	Inf.	Ins.‡ 5–2
1	5	§	5	+	5	5	+	2	+	0
2	5	+	5	+	5	5	−	3	−	1
3	5	−	5	+	5	5	+	2	−	0
4	5	+	4	+	4	5	+	4	+	0
5	5	−	5	+	5	5	+	3	+	1
6	5	−	5	+	4	5	+	4	+	0
7	5	+	5	+	5	5	+	4	+	2
8	5	+	5	+	5	5	+	4	+	2
9	5	+	5	+	5	5	+	4	+	2
10	5	+	5	+	5	5	+	2	+	2
11	5	+	5	+	5	5	−	4	+	1
12	5	−	5	+	5	5	+	5	+	1
13	5	−	5	+	5	5	+	4	+	0
14	5	+	5	+	3	5	−	3	+	1
15	5	+	5	+	5					
16	5	+	5	+	5					
17	5	+	5	+	5					
18	5	−	5	+	5					
Totals	90	11/18	89	18/18	86	70	11/14	48	12/14	13

Ins = insects Inf = infections
† Number of insects in each colony [illegible] fed on first test plants [illegible] one week [illegible] second plants for two weeks
‡ [illegible] colony at end of second test feeding
§ Pl[illegible] test plant became diseased [illegible] test plant remained healthy

insects with dilution is not an exception but is due to incomplete presentation of the data in summary form

Although Bawden[3] (page 95) contended that this technique was inadequate to demonstrate differences in virus concentration he (page 96) accepted the data (TABLE 4 and other data) as indicating the source (insects that had fed all their lives on diseased asters) used to inoculate control insects was richer in virus than the insects being titrated (insects that had fed on diseased asters for one day) The virus content of the latter source was lower than that of the former because a smaller proportion of them had picked up virus This was indicated by the fact[5] (page 130) that only two out of 50 insects in one of the latter groups transmitted the virus when tested individually for two days on asters Kunkel[23] in extensive experiments has shown that many of the insects

do not pick up virus after one day of feeding on diseased plants and some do not pick it up even after one week of feeding on diseased plants

The general validity of the technique used in the titration experiment for obtaining a measure of virus concentration has been established by experiments on wound tumor virus[9] reproduced in TABLE 7 Here the logarithms of the percentages of infective insects when plotted against the logarithms of dilutions 10^{-2} to 10^{-4}, were found to fall close to a straight line

To my knowledge, the only explanation other than multiplication for the results of these experiments is the one mentioned in the original publication[9] dealing with them namely that the virus may be present in more readily dispersed form at one time in the incubation period than at another It should be remembered however that there is no evidence to support this interpretation

Incidentally Kunkel[17] has pointed out the existence of an interesting correlation between the lengths of the incubation periods in the insect and the plant for a number of viruses

Experiments on Serial Passage of Aster Yellows Virus through a Vector The technique of transferring the virus by injection into leafhoppers was used by the writer in each of the four years 1939-1942 to carry out an experiment on serial passage from vector to vector on a large scale Only negative results were obtained More recently however, Maramorosch[25] has succeeded in using the insect injection technique described above to pass aster yellows virus from vector to vector in series without replenishment of virus from plants. Starting with 100 viruliferous aster leafhoppers weighing 140 mg he employed a microsyringe to inject virus free insects with measured amounts of known dilutions of the source leafhoppers It was calculated that the virus was diluted approximately 10^{-4} at each passage Between passages the injected insects were kept on caged plants provided with excellent illumination at a constant temperature of 25 C This was probably important in the success of his experiment During the first six passages the injected insects were kept on rye which is immune to the virus for 30 days and the survivors then were tested individually on susceptible asters for two days before being used as a source of virus for subsequent passages During the last four passages the insects were kept for long periods on asters and were transferred to fresh healthy plants three times a week, in some cases and every five days in the other cases Virus could not have been replenished from the rye plants fed on by the insects because rye is immune

The immunity of rye to aster yellows virus which was established by Kunkel [18] has been repeatedly tested and was retested by Maramorosch in these experiments The rye plants never develop symptoms of the disease and virus cannot be recovered from plants on which infective insects have fed Virus could not have been replenished from the aster plants fed on by the insects in the series because Kunkel [2] and Maramorosch[25] showed that the minimum incubation period in asters was nine days and Maramorosch [5] showed that insects could not recover virus from plants until the seventh day after inoculation Despite Storey s evidence of rare recovery of maize streak virus by non

viruliferous vector insects feeding on the same leaf as infective insects during periods of one or two days it seems most improbable that the insects in Maramorosch s experiment obtained aster yellows virus from plants during the course of the experiment The dilution of original virus used for injection in the tenth passage was 10^{-40} (TABLE 6) yet tests indicated that there was just as much virus present at this passage as in the first passage The successful inoculation therefore attained in the tenth passage with a final dilution of 10^{-40} in terms of the original virus provides convincing evidence that the virus multiplied in the insect

Experiments with Wound Tumor Virus Maramorosch s successful serial passage of a plant virus through its leafhopper vector has been confirmed by Black and Brakke[9] who used wound tumor virus and a somewhat different

TABLE 6

SERIAL PASSAGE OF ASTER YELLOWS VIRUS THROUGH THE ASTER LEAFHOPPER

Pas g	N f I j ct d sect	D y ft on j t		S rv rs	I f t rv	C t l t	C lcul ted d l t
		Ry	A t				
1	200	30	2	4 36	8	100	10^{-4}
2	300	30	2	16	3	100	10^{-8}
3	150	30	2	2	0	100	10^{-12}
4[b]	300	30	2	34	5	100	10^{-16}
5	100	30	2	5	0	100	10^{-20}
6	200	30	2	22	5	100	10^{-24}
7	100	6	54[d]	*B* 8	2	20	10^{-28}
8	240	40	25	12	4	20	10^{-32}
9	50	19	13	16	6	20	10^{-36}
10	50	30	0	4	1	20	10^{-40}

Aft M ram ros h
[b] Th d l t d t l th f th pas g w filt ed th gh int ed gla
P ill w dded th f urth fifth th d eventh p ges
[d] I d dual in ects w tra f ed t d d l ast plants th ee t m weekly
Ind d l se t w tr f rr d to d vid l t pla t ry fi day

experimental plan In their experiments the original source of virus was 60 adult leafhoppers of the species *Agallia constricta* Van Duzee An extract of these leafhoppers was prepared in a buffered saline solution and various dilutions of it were injected into virus free leafhoppers Most of the injected insects were kept on immune Grimm alfalfa for two weeks and then tested individually for infectivity on susceptible crimson clover plants for one month Other insects injected with the more concentrated solutions were kept for a month on alfalfa plants in cages free of weeds These insects were then used as a source of virus for succeeding passages Using the weights of the leafhoppers the total volumes of the dilutions injected and the proportions of the injected insects used as a source for further passage it was possible to calculate the dilution of the original source of virus at each transfer The results obtained during seven passages are summarized briefly in TABLE 7 It is obvious that the original virus source was diluted to about 10^{-18} whereas the concentration of virus in the insects at each passage attained approximately the same level

The immunity of alfalfa to wound tumor virus was carefully tested[9] Plants were grafted with pieces of tumor induced by the virus in sweet clover *Melilotus alba* Desr Such plants failed to develop symptoms of the disease after eight and one half months and vector insects fed on them failed to pick up virus In other tests genetically marked infective female leafhoppers were kept on the same alfalfa plant with virusfree females of a different marking Although the infective and virusfree females and their progeny fed on the same plant, only the progeny from infective females proved infective The experiments proved conclusively that a small percentage of progeny received virus from their mothers through the egg and provided evidence that the alfalfa was immune Most of the alfalfa plants used in the experiment were themselves tested for virus by means of virusfree insects and in two separate tests the

TABLE 7*

SERIAL TRANSMISSION OF WOUND-TUMOR VIRUS THROUGH AN INSECT VECTOR (*Agallia constricta*) GROWN ON IMMUNE ALFALFA

Passage	Infectivity of insects inoculated with insect juice at indicated dilution							Fraction of [illegible] (m [illegible] g)	Uninoculated control insects
	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]	10[illegible]		
1	0 24†	0/21	0/17	1/20	1/20	6/20	9/30	0 00	0/30
2	—	—	0/28	1/28	5/27	—	6/28	3 06	0/27
3	—	—	1/16	3/20	4/20	—	4/10	6 05	0/20
4	—	—	—	0/20	3/27	11/27	—	8 46	0/24
5	—	—	—	1/27	5/29	11/30	—	12 13	0/25
6	—	—	—	0/25	8/28	15/28	—	15 24	0/27
7	—	—	—	2/30	8/28	17/30	—	18 32	0/28
Total infective	0/24	0/21	1/61	8/170	34/179	60/135	19/68	—	0/181
Percentage infective	0	0	1 6	4 7	19 0	44 4	27 9	—	0

* After Black and Brakke
† Numerator is the number of insects infected, denominator the number of insects tested

results indicated their immunity to the virus These experiments provide convincing evidence for the multiplication of wound tumor virus in one of its insect vectors

The logarithms of the percentages (TABLE 7) of infective leafhoppers inoculated by injection were directly proportional to the logarithms of the virus concentration Only the results with dilution $10^{-1.5}$ were somewhat out of line The method used in this experiment was essentially the same as that used by Black[6] in his titrations for increase of aster yellows virus during the incubation period in the leafhopper

Studies on Curly Top Virus (*Ruga verrucosans* Carsner and Bennett) It is necessary to consider the bearing that experiments on curly top virus[12, 4] have on this whole question Bawden has placed much emphasis on this evidence in his treatment of the problem It is quite possible that curly top virus does not multiply in its insect vector *Circulifer tenellus* (Baker) while a number of other leafhopper viruses do It is true that the ingenious and extensive experiments of Freitag and of Bennet and Wallace failed to provide evidence for

multiplication of curly top virus in its vector Their results justify the statement of Bennett and Wallace[4] that if there is any multiplication of the virus in the leafhopper it is not sufficient to maintain the original virus content The possibility however that curly top virus undergoes limited multiplication in the insect either systemically or in local lesions and is subsequently lost was not eliminated and this is a most difficult possibility to resolve It should be recognized that certain lines of evidence such as the loss of infective ability by insects irregularity of transmission or even failure to transmit during the whole life of the insect are not critical so far as the question of multiplication of virus in the vector is concerned because these phenomena occur with some of the above viruses demonstrated to multiply in their vectors It should be remembered also that many animals recover from repeated infections by the same virus

Discussion Of the four agen discussed above which grow in their insect vectors only two (those causing aster yellows and wound tumor) have been passed through filters that will retain small bacteria[8, 10] There is little doubt that all of them are viruses Sedimentation studies of the wound tumor agent clearly place it in the size range of the viruses[10*] These agents have long been considered viruses by plant pathologists on the basis of the symptoms they produce i plants and their transmission by insects or by grafts No efforts to cultivate them on cell free media or to identify them under the light microscope have been successful Their ability to multiply in plants has rarely been questioned The aster yellow and wound tumor viruses have been transmitted by grafting as a matter of convenience in numerous experiments and clover-club-leaf virus was passed through 30 serial passages in periwinkle (*Vinca ro ea* L) without the intervention of insects[8]

The different types of evidence obtained in studies on rice stunt aster yellows clo er club-leaf and wound tumor leave no doubt that these viruses multiply in both plants and in ects Because leafhoppers suck up plant juices and not intact plant cells multiplication of the viruses in host plant cells in the insects intestinal tracts seems most improbable Moreover in some of the experiments cited above p art cells involved are immune to the virus in question Viruses are not known to multiply in cellfree media and there i no reason for assuming that the e do The only logical site for their multiplication is in the cells of the insect or in certain structures presumed to be symbiont that are commonly present in certain inse t cells At present there i no evidence that would resolve this last question Whether these viruses multiply in the insect cells or in the presumed symbionts however they have been shown to grow in more widely separated organisms in the phylogenetic sense than any other viruses They either bridge the gap between plant viru es and animal viruses or the gap between plant viruses and those of microorgani ms

It seem probable that these viruses are actually both plant and animal viruse that the specificity of their insect transmission is determined in part

* M ent work by M K B kke, A. E. V ll d L. M. Bla k (to be p bli hed) has demon tr ted that w nd-tumor ng d ap bo t 80 mμ in d met

at least by their ability to multiply in the insect in question and that the incubation period in the leafhopper is in part, a *measure of this multiplication* There would seem to be little doubt that multiplication of the virus in the vector explains the frequent ability of these insects to remain infective through out their whole life once they begin transmitting virus

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Discussion of the Paper

F C BAWDEN *Rothamsted Experimental Station Harpend n England* Some people had unhesitatingly concluded from earlier work that certain plant viruses multiply in their insect vectors To them Dr Black s monumental study of clo er club-leaf virus in *Agalliopsis novella* may seem less impo tant than it does to those like myself who consider that all the earlier work had other equally plausible interpretations His were the first results that seem un equivocal but no w as he has shown in his paper there is convincing evidence that clover club-leaf is not the only virus that multiples in both plants and insects This fact is relevant to the subject of our monograph for two differ ent reasons First it demonstrates clearly the artificiality of erec ing taxo nomic barriers between viruses simply because they are found in different kinds of organisms and it emphasizes the inadvisability of adopting any separate systems of nomenclature or classification for the viruses that are studied in animals bacteria or flowering plants Secondly it illustrates the difficulties in eparable from any attempt to derive a 'natural or phylogenetic cl ification of viruses for it shows that viruses may have originated in sources remote from those in which they are currently most important and most studied On the a sumption that pathogens will often be most virulent in recently acquired habitats I have previously suggested that plausible sources of plant viruses lie in the insect pe ts that are their symptomless vectors, but that thi origin could not be seriously considered until there was good evidence that these insects ould support the multiplication of the viruses they transmit Dr Black has now provided this important evidence

PROBLEMS OF VIRAL NOMENCLATURE AND CLASSIFICATION

By Francis O Holmes

The Laboratories of the Rockefeller Institute for Medical Research New York N Y

Classification of viruses would be a much easier task than it is today or than it has been in the past, if all the viruses that have been investigated were equally well known The fact is however, that some viruses have been studied both extensively and intensively, whereas others rarely have been subjects of research since they were first discovered Adequate reasons exist of course to account for the discrepancies Viruses have been studied more or less in proportion to their individual availability, economic and public health significance to man and ease of manipulation in the laboratory In some cases one factor has been decisive In other cases combinations of factors have influenced the amount of research

It is obvious that unequal availability of viruses for study has caused some inequalities in our present knowledge about them A few viruses have attained such widespread distribution as to be found now in every continent Others still occur only in more or less restricted areas Naturally those characterized by wide distribution have been available for repeated studies by many investigators In most cases those of narrow distribution have been studied less intensively and much less often though there are notable exceptions such as maize streak virus in the plant-disease field and myxoma virus in the animal disease field

Great differences in the economic and public health significance of individual viruses naturally have had profound effects on research Widespread epiphytotics epizootics, and epidemics have encouraged investigations of some viruses such as equine encephalitis and influenza viruses in the animal and human disease fields and spotted wilt virus in the plant-disease field The relative insignificance of losses caused by certain other viruses has discouraged their study However natural this may be it is to be regretted at least in those cases in which a lack of balance within the field of virology has been a result

Adaptability to the techniques that have been used thus far in research has characterized viruses very unequally Properties that have facilitated use in laboratory experimentation have tended to make some viruses well known As examples may be cited tobacco mosaic and tobacco necrosis viruses among those causing disease in plants and influenza vaccinia and poliomyelitis viruses among those affecting animals and man Properties that have tended to limit usefulness for exhaustive studies have discouraged investigation of some other viruses As examples may be mentioned locust witches broom virus in the plant disease field and human wart virus in the human disease field

Perhaps in part because of the unequal study of individual viruses, natural groups did not become recognizable for some years after study of individual viruses was begun Gradually however, it became evident that viruses having one characteristic in common tended to have other characteristics in common

also This concept supplied a basis for attempts to formulate a natural system of classification

In the plant disease field a large proportion of viruses that produce virescence in flowers are known to have leafhoppers of the family Cicadellidae as their insect vectors All viruses of this group that have been tested are characterized also by low thermal stability They agree in consistent induction of sterility in affected host plants In most of their hosts they tend to break the dormancy of axillary buds They usually prevent the development of normal pigments in affected flowers though the flowers may contain chlorophyll in parts not normally green In almost all hosts affected leaves become uniformly chlorotic although occasional host plants remain relatively green In most host plants also affected branches assume a more nearly vertical position than is normal although exceptional species are much less affected than the average

Viruses that produce mottling type diseases in plants are characterized on the other hand by aphid vectors These aphid transmitted viruses are more stable to heat than are viruses of the above mentioned leafhopper transmitted group They do not break the dormancy of axillary buds nor do they induce sterility or virescence in flowers Affected branches continue to form approximately normal angles with the stem Affected foliage is not uniformly chlorotic but typically is mottled though veinal markings or necrotic lesions may appear or there may be an almost complete lack of obvious disease in special cases

The two natural groups of plant disease viruses thus suggested have come to be known by names of induced diseases Thus we speak of the yellows disease group and of the mosaic disease group of viruses These groups however are not differentiated solely or even principally on the basis of induced symptoms Like all other viral groups they are based on the taxonomic concept of type Each small group is formed by assembling around one virus designated as type of its genus all other known viruses that closely resemble it Those not at all closely resembling the type of one group are taken to be types or to be members of other genera similarly formed Larger groups are formed likewise by assembling around one genus taken as type of its family all other recognized genera that clearly resemble it

The probability that any virus assigned to a supposedly natural generic group has been correctly placed there may be estimated in general by the proportion of its characteristics that agree well with the characteristics of the virus that was taken as type for the genus The fact that much has been learned of induced diseases and relatively little about some other characteristics of the viruses has led of course to an undue conspicuousness of information about types of induced disease and in the plant-disease field it is only the information about arthropod vectors that has in any way rivalled this body of information

A generalization firmly established for plant-disease viruses is the mutual exclusiveness of transmission by aphids leafhoppers white flies and thrips No single virus is known to be transmitted by two of these four vector classes A few viruses it is true have properties such that a variety of insects transmit

them more or less incidentally For example, potato spindle tuber virus is transmitted by grasshoppers beetles, and true bugs as well as by aphids. This virus is transmitted also by knives used in cutting seed pieces from tubers and by simple contacts between the freshly cut seed pieces The high concentration of this virus in plants that it infects probably explains transmission by some of the leaf-eating insects and by mechanical contacts The fact remains however that evolution of many other viruses seems to have been largely dependent on their association with such insects as aphids leafhoppers white flies or thrips A high degree of specialization for these vectors has been developed Evidence has been found that some viruses that multiply in plants and produce diseases in these hosts also multiply in their insect vectors although they have not been shown to produce obvious diseases in the insects We cannot be sure that investigations in the future will fail to disclose viruses that can be transmitted both by aphids and by leafhoppers, by white flies and by thrips or by some other combination of two types of vectors in the four groups that have been mentioned We have only the knowledge that some claims concerning pairs of such vector types have been made in the past but that all of these thus far have not been confirmed on more careful investigation It seems probable that such pairing of vector types if it exists is a rare and exceptional occurrence and not at all a usual phenomenon On this account it seems feasible in attempting classification of a virus to place considerable dependence upon the type of vector, when this is known

Unfortunately we do not know the arthropod vectors for nearly half of the recognized plant-disease viruses In contrast to this, something is known of natural or experimental induced diseases in all cases We need not conclude that the viruses for which no vectors are known actually lack insect vectors in nature In some cases technical difficulties may have delayed the discovery of appropriate vector species In others little or no effort may have been made to identify vectors Naturally it has been necessary to depend on whatever other information was available to assign the less well-characterized viruses to appropriate groups pending discovery of their vector relationships

The attempt to classify some viruses according to their natural relationships without the benefit of information on their insect vectors, has repeatedly permitted predictions as to the type of vector that might be sought with the greatest chance of success Before the vector of tomato big bud virus was known it was clear that a cicadellid leafhopper should be expected to be its transmitter This was because tomato big bud virus was regarded as belonging to the true yellows-disease group and all viruses known to be transmitted by insects in this group of viruses had been shown to have cicadellid leafhoppers as their vectors The vector subsequently found for this virus was *Thamnotettix argentata* Evans a cicadellid leafhopper as had been expected Many attempts have been made to find a vector of sandal spike disease virus in India This virus also appears to belong in the true yellows-disease group In recent years two cicadellid leafhoppers have been suspected of transmitting it In both instances the number of observed transmissions has been small and further studies might well be made It may be predicted however that one or both of these suspected species or at any rate some cicadellid leafhopper or

leafhoppers will be found on further study to be the natural transmitters of this virus In North America locust witches broom viru and potato witches' broom virus have as yet no recognized vectors but also are regarded as members of the typical yellows-disease group It may be predicted with considerable confidence that they are capable of being transmitted by some species of leafhopper almost certainly of the family Cicadellidae Should they be reported as being transmitted by an aphid a white fly or a thrips we should be justified in concluding that the report requires further investigation before being accepted at face value In fact we might conclude tentatively that the report was probably in error

This possibility of using our growing knowledge of viral relationships as a guide to research through prediction of vector types and some other characteristics of viruses is one of the most valuable gains from natural classification and repays in part at least the expenditures of effort and patience that are required to deal with the many problems till remaining in this field of investigation It may be admitted freely that errors in our present concepts of natural relationships may lead occa ionally to predictions that will not be ju tified by subsequent investigation Each uch outcome however may be expected to lead to improvements in our attempted classification thus increasing the probability of correct predictions in future work

Many of the troublesome problems of viral taxonomy arise from our still limited knowledge Can we overcome the hand caps imposed by differences in availability economic and public health significance to man and ease of manipulation of individual viruses? If not we must expect to continue to experience and tolerate some inequalities in development in different parts of the field covered by the science of virology

A much larger number of viruses than we recognize today probably will become known in future years Groups of which we now know many representatives will then be still larger and perhaps will come to be ubdivided into easily recognized subgroups This in all probability will be the fate of the mosaic group among plant-disease viruses and the pox group among animal disease viruses Sub tantial groups of close relatives may come to be recognized as all ed to most of the viruses that seem to stand alone at the present time although ome may remain isolated as evolutionary remnants It is not ea y to refer potato spindle tuber virus to any large group today but the discovery of other viruses having properties more or less similar may on the whole be anticipated Potato pindle tuber virus may thus come to be looked upon as an early known representative of a temporarily neglected natural group In the same way potato leafroll and sugar beet yellow viruses stand somewhat apart from others that are aphid transmitted They do not induce typical mottling type diseases as many aphid transmitted viru es do Other viruses similar to these may come to be recognized in the near future and it may then seem s rprising that so little study was given to such a group of viruses in the course of earlier years Rabies virus seemed to stand alone in an earlier view but a recent revision by Ansell associates it with three other viru es those causing Aujeszky s disease Borna disease and Bwamba fever

A few viruses that are regarded today as distinct from each other probably

will be recognized in the future as closely related strains of single viruses This happened recently in the case of citrus quick-decline virus of North America tristeza virus of South America, lime disease virus of the Gold Coast in Africa and grapefruit stem pitting virus of South Africa which seem to represent merely geographic strains or isolates of a single viral species[11] Such occurrences may oppose the trend to larger numbers of recognized species but they are unlikely to reverse it For many years the recognition of groups of strains has been highly satisfactory for most of the viruses that are capable of acting as good antigens when injected into the blood stream of animals such as rabbits Serological identification of related strains has been supplemented by immunological studies based on so-called protection tests in which initial infection by a relatively mild strain of virus protects against subsequent increased damage on inoculation of already invaded tissues with a severe symptom strain of the same virus It is still an unsolved problem whether viruses that have appeared to lack antigenicity can be treated in such a way as to obtain specific antisera If no progress can be made in this direction there is still the possibility that some process may be found to supplement the serological and immunological procedures in the identification of viral strains In some measure the study of extensive experimental host ranges has filled such a need for it has been observed that known related strains tend to have identical or almost identical hosts whereas unrelated strains ordinarily differ sharply in this respect

New types of viruses and as yet unrecognized kinds of arthropod vectors may be found in the future These may tend to bridge the gaps that separate some natural groups at present We may confidently expect that a recompense for any confusion caused by the discovery of novel types of viruses or vectors will be found in the increase in adequacy of the available information and in the consequently more complete classification that can be attained Obviously some groups will appear unrelated so long as we remain in ignorance of intermediate types

It has been pointed out more than once that the desirability of the binomial form of nomenclature for viruses should be considered without respect to the appropriateness or lack of appropriateness of attempted classifications Modification of tentative groupings formation of new groups and perhaps elimination of old groups can be put into effect whenever they seem justifiable Binomial nomenclature has amply proved its value for representing changing views of natural relationships

Investigators can assist the effort to assign new viruses to appropriate places in the taxonomic structure whether or not they themselves desire to take any active part in the classification of viruses if they will provide distinctive common names both for the viruses that they may study and for the diseases that these viruses induce A difficult problem is presented when an investigator refers in a preliminary paper for example merely to a new virus affecting one or another host species If no binomial is applied failure to suggest so much as a common name leaves a new virus without any differentiating appellation under which it can be discussed As a minimal requirement a dis-

tinctive disease name greatly facilitates further handling To choose an example from the plant-disease field the suggestion of such a name as peach phony disease permitted the causative virus to be discussed under the name 'peach phony disease virus whereas a paper referring to a new virus causing abnormal growth habit in peach trees would not provide for the construction of a common name for the causative virus It is less important that such a name should be appropriate than that it should be of a form adaptable to use for the causative virus and especially that it should be distinctive When one disease has been called pea mosaic it is hardly sufficient to refer to a second viral disease of somewhat similar characteristics as a new pea mosaic disease because the causative virus is not thus provided with a distinctive common name It would be better if some other feature of the new disease could be indicated It is feasible to call the new disease pea mottle pea streak or as has been done in one case pea enation mosaic Each of these disease names provides for the formation of a distinctive common name for the causative virus

A problem that disturbs some who have given attention to the taxonomy of viruses has to do with what now seems to be an artificial separation between groups of viruses that attack higher plants those that affect animals and those that attack bacteria The viruses causing diseases in higher plants were studied primarily by plant pathologists those affecting animals by veterinarians and medical investigators and those affecting bacteria by bacteriologists Lack of known relationships between viruses of these three great classes may be attributable in part to lack of common background among their investigators Probably not enough is known yet about resemblances between individual viruses in these separate groups to predict whether there may be taxonomic overlapping when the groups are better known It must be pointed out however that these groups may remain separate indefinitely Their evolution has been dependent on such distinctive hosts that repeated mutations and selections in them could have produced profound effects Little evidence might remain of any relationships that possibly existed between them in the remote past

Geographic concentration of investigators probably has led to a distorted concept of the range of variation in viral types If North America were as little studied for viral diseases as is most of Asia we should know but little of the now well studied yellows-disease group of viruses What groupings will be equally obvious when the Asiatic viruses have been thoroughly studied we cannot now predict If the continent of Africa had not been the site of extensive investigation of viral diseases we should have only a little knowledge of white fly transmission of viruses which has been investigated elsewhere only in Asia and South America Future studies in Central America and in northern South America may disclose as yet unimagined types of vectors and of viral diseases

Viruses that have been studied extensively to date have in most cases forced themselves on the attention of investigators by their destructiveness It is not surprising if our knowledge of the field is consequently only partial

Such viruses as Bennett's dodder latent mosaic virus which induces no obvious disease in the only recognized natural host *Cuscuta californica* Choisy, and Theiler s mouse poliomyelitis virus, which produces spontaneous flaccid paralysis of hind legs in its hosts only rarely may be more common than we realize at present

The active study of viruses has been carried on now for a little more than fifty years In contrast to this attempts at recognition of natural groups among viruses have been relatively recent It seems reasonable to suppose that improvements through revision of existing classifications may require many more years than have already elapsed If however revisions continue to appear at the present rate progress should be rapid Earliest revisions of course were largely in the plant-disease field These were published by W D Valleau University of Kentucky,[10] H H McKinney, United States Department of Agriculture[4, 5] Eubanks Carsner and C W Bennett, Riverside California[2] and Pierre Limasset, Versailles France[3] Later revisions and additions in the animal-disease field were published by David Sompolinsky Copenhagen[7] Edward A Steinhaus University of California[8, 9] I A Merchant and R A Packer Iowa State College[6] and M Ansel, Paris[1] A continuation of this series of revisions without decrease of average quality will ensure steady improvement

It is not at all essential that classification of viruses in natural groups should be entirely accurate and successful from the first Perhaps however, some adverse criticism of what has been accomplished thus far has been based on a vague feeling that success can be, and should be achieved at once If in first attempts however two dissimilar viruses are grouped together this fact will soon come to the attention of specialists working with them and there will be a tendency for the mistake to be corrected Even if two similar viruses are separated in their initial classification a less easily recognized error the discrepancy is likely to be noticed sooner or later and an improvement of classification introduced through revision It would seem unnecessary and impractical to expect complete and correct treatment of all natural groupings on their first formulation

Repeated revisions may be needed to attain a reasonably satisfactory delineation of groups of viruses as they occur in nature The attention of investigators however has been focused now on similarities and dissimilarities among the viruses with which they work This has been a natural result of the early attempts at scientific nomenclature and classification for viruses It was what had been anticipated and desired

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THE NATURE OF VIRUSES IN RELATION TO NOMENCLATURE

By James Johnson*

Departments of Horticulture and Plant Pathology University of Wisconsin Madison Wis

The need of a satisfactory system of virus nomenclature has interested our laboratory since 1925 at which time the use of a numbering or lettering system for indexing the plant viruses was suggested In the following years a number of systems were proposed by various workers These have been summarized and discussed in an article published in the Tijdschrift over Plantenziekten in 1949[7] The conclusion reached in that review was that we need to know more about the true nature and relationship of viruses before we proceed further in classification and nomenclature This is still the situation and consequently I wish to devote this paper to a discussion of the nature of viruses

Although we have numerous discussions and reviews on the nature of viruses few data are aimed directly at a solution of the problem This may have resulted indirectly from the complexity of the subject and the confusion in our minds that has arisen from the various viewpoints presented in the last two decades Perhaps we lean too greatly on the enormous contributions of chemistry physics genetics mathematics *etc* to our understanding of the characteristic of the viruses It is possible that all these details may lead us astray from the over all analysis of the entire complex

The early background of the subject is suggestive in this connection If we conceive of a brilliant scientific mind centered on a new subject, but unconfused by many perhaps irrelevant, later opinions we have a situation *worthy of our consideration Such a man in the field of virology was the noted* Dutch botanist and bacteriologist Dr Martinus Willem Beijerinck His paper on a Contagium vivum fluidum as the cause of tobacco mosaic published in 1898[2] should be read by everyone interested in the nature of a virus His experiments were convincingly conducted and remarkably modern in conception and performance His reasoning therefrom was clear and reliable and his publication is a classic today He was satisfied that the mosaic disease was not caused by microbes but by a contagious living fluid These conclusions have not been far advanced to this day We would all do well to stop and consider how Beijerinck would react to our present theories on the nature of a virus now that we have more of them

Another early worker and thinker on this subject whose opinions we cannot afford to ignore at this critical stage in nomenclature, is Erwin Bauer who later was recognized as an authority on genetic research Publishing in 1904 on the etiology of infectious variegation ' he came to the positively stated conclusion that there is a typically infectious disease for which living organisms cannot be considered as causative agents [3]

A similar conclusion has become obvious to numerous other workers with a variety of plant and animal viruses since that time That living microorganisms may be so small as to be invisible under the light microscope is readily

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conceivable but leads to no satisfactory conclusion in view of other known facts about virus behavior It is quite as likely that some living particles are invisible under the electron microscope

It is not implied that we are in a state of unnecessary confusion regarding the nature of viruses at the present time All that is definitely known is basic to a final solution of the problem The rapid increase in the number of described viruses and the differentiation of viruses and virus strains is for example essential to the correct understanding of their nature In other words it is not sufficient to understand the tobacco-mosaic virus or the small pox virus only An explanation of their nature must fit all the true plant viruses or all the true animal viruses or better still both virus groups It is quite inconceivable that we are dealing here with two or more separate and distinct classes of disease in view of their numerous similarities

When discussing a virus it is necessary to regard it as infectious and if infectious as containing living matter Beijerinck himself showed that an extremely small amount multiplied in one plant and subsequently transferred to other plants could increase to enormous amounts Reproduction and growth were evidence of its living nature Whether or not the fluid contained particulate bodies was not clearly established from present-day points of view Though microbes could be eliminated and the filtrates were obviously sterile on any synthetic media that were used the obligate parasites to be found among the higher organisms demonstrated the need for caution

The electron microscope opened a new field for research and speculation Certainly there were rod-shaped particulate bodies in the tobacco mosaic virus which because of their small size could pass through bacteria proof filters but which could not be cultured on synthetic media Many shapes and sizes of particles have been reported in many other viruses as by Wyckoff in 1948[3] Conclusive proof that such particles are the causal agent rather than the result of the disease apparently remains to be established[4]

The chemical hypotheses range over a considerable area of conceptions as to the type of virus activity Studies on virus purification or purified virus proteins have occupied most of the attention of the chemists It would be difficult to conceive of virus except as proteinaceous particles or molecules of protoplasm or cytoplasm either of the host or of foreign parasitic origin Some disagreement may be expected as to the actual purity of a virus or its freedom from other forms of protein or living matter since purification relies on separation by centrifugation precipitation or other methods not completely satisfactory[1]

It is significant however that the trend of the chemical investigations has been developing more and more toward the host protein relations and the conclusion of several plant virus workers has had a stimulating influence on our thoughts on the subject In the animal virus field as well workers such as D J Bauer[4] have found evidence that viruses may be formed by the conversion of normal cell constituents

In view of recent trends of thought I should like to present the viroplasm hypothesis in this paper[5] This is an unusual opportunity to obtain criticisms

and comments, which have been altogether too rare in the quarter of a century since this hypothesis was first promulgated

First of all it may be taken for granted that any other satisfactory explanation for the origin and nature of viruses would naturally eliminate further consideration of the viroplasm hypothesis Consequently, major research and thought have been given in recent years, to the other hypotheses, especially those that involve a physical and chemical explanation of the observed facts As far as we can see into the future of the subject there is room for the viroplasm conception, which fits an unusual number of facts that are known about viruses and which has "few bridges to cross" albeit some involve unknown or uncertain lines of biological behavior

The viroplasm hypothesis assumes that certain cytoplasmic components of normal or healthy individuals, especially living protein molecules, may be introduced (as by sucking insects) into certain other normal individuals usually related forms and, when certain conditions involving mutual compatibility are fulfilled may combine with the host cytoplasm multiply, and thereby incite an abnormality within the cell, as, for example cytoplasmic inclusions They may be capable therefore, of yielding symptoms in some instances and, most important may be capable of transference to other susceptible hosts by the proper means

That cytoplasmic combination or adsorption occurs to a remarkable degree is illustrated by the numerous instances of virus inactivation *in vitro* by foreign proteins Some proteins do not inactivate others and still others create a host reaction which although it creates a systemic disturbance comparable to an allergic response is not capable of continued transfer The actual synthesis of a typical virus disease by a combination of known normal cytoplasmic components has not been positively accomplished The detail and routine work necessary to such attempts is obviously great considering the number of possible combinations and the possible methods of transfer that may be used in bringing compatible cytoplasm together within individual cells Furthermore the origin of some of our common viruses may be obscured by the accidental proximity of now extinct species rendering the experimental demonstration impossible

The initial successful union of plasms needs to be a rarely occurring phenomenon, otherwise virus diseases would be rampant The viroplasm concept fits the known conditions and requirements of a virus such as invisibility or small size filterability, molecular protein structure, physical and chemical properties dormant persistence and renewability as in seed and vegetative parts used in propagation *etc* Most important is the inheritable stability of a virus In this respect it behaves like bacterial cells spores seeds or cells in general Minor variations or strain differences occur in each individual virus highly suggestive of higher forms of living matter One of the many favorable features viroplasm possesses is that it need not assume the *de novo* origin of a virus though it must admittedly, still hurdle the barrier of subsequent transfer by means other than graftage

There are many common phenomena in nature that basically seem quite as

obscure as the viroolasm hypothesis Though the behavior of inheritance or the result of sexual fertilization is well understood the union of the germ plasm and subsequent growth often from two different species is fantastically complex and unpredictable The union of cytoplasmic molecules perhaps is more easily compared to asexual unions such as occur in graftage chimeras symbosis parthenogenesis allergies *etc* Much of the support however for the behavior of normal cytoplasm may be found in virus behavior itself once we have overcome the dogma in pathology that independent organisms are the basis of all infections

Although we have made at least one serious attempt to synthesize a virus disease by transferring normal cytoplasm from 122 species representing 50 genera of legumes to the common bean by the mechanical means the mathematical probabilities of realization seemed too remote and it was discontinued We did however secure a response in 100 per cent of the trials with Tangier pea to bean but this was finally interpreted as an allergic response

Recently we have attempted a method of attacking the problem that is comparable to a method of analysis rather than synthesis of the component factors inducing different types of symptoms

For this purpose we have used the well known and widely studied yellow tobacco-mosaic virus strains These strains are regarded as mutants of a green mosaic strain though the frequency of such mutations is questioned and the origin of the strain may be more remotely obscured in the past than has been commonly believed This virus strain may be regarded in any case as a typically good virus in its characteristics and behavior It is somewhat unstable in the sense that the yellow symptom expression is varied by a number of circumstances especially temperature light and interference phenomena

For many years we have been puzzled by the observation that we have never been able to secure a yellow strain that yielded yellow symptoms only despite innumerable single lesion isolation cultures Mild green symptoms always developed prominently under favorable conditions for their expression The frequent uncertainty and unreliability of single lesion isolations are recognized and considered but paradoxically it is clear that many other pure strains of the tobacco-mosaic virus may be isolated quite easily in this manner For example a green strain of virus may be readily freed from the yellow symptom in this manner though absolute freedom from the yellow character is not always easily demonstrated except by especially favorable hosts and environment for its expression The common yellow strains as well as one or more of the mild green strains regularly produce local necrotic lesions on *Nicotiana sylvestris* and not on *Nicotiana acuminata* One of the yellow strains however yields only systemic symptoms and not local lesions on *Nicotiana sylvestris* Separation of the mild green and let us say common yellow types of symptoms are therefore apparently not separable on *Nicotiana sylvestris* on the basis of necrotic reaction alone as was formerly believed Using a combination of host susceptibility and environment however it is possible to isolate a mild green strain from the yellow factor though the yellow factor cannot be separated from the green strain We have done this frequently on *Nicotiana*

tabacum that reacts systemically to both the strains in question If the yellow types are grown at 40 C for three to six or more days soon after inoculation, the yellow factor will remain localized and the green strain will become systemic in the new leaves and consequently, may be freed from the yellow factor by transfer It should be explained that after systemic invasion has occurred, a temperature of 40°C is by no means unfavorable to the yellow factor This sounds suspiciously like simple strain separation or induced mutation It would be superficial to assume mutation here since the green strain was already present The yellow factor is eliminated Hence we are convinced that two components rather than strains were present in the beginning The yellow symptom is a factor or precursor that must be associated with the green strain in order to multiply and express itself The method I have described is not the only way that such separations occur They may be noted in the greenhouse and in nature, but complete separation free from possible mixture of virus and the yellow factor, is not easy to assure although they are partly responsible for the apparent higher variability of this strain of tobacco-mosaic Altogether it seems possible that the yellow form apparently mutates to the green more frequently than the green form does to the yellow but mutation should not be assumed when separation is a logical explanation Furthermore it seems reasonable to believe that we do not actually have a pure yellow strain but that the factor that produces yellow is only associated with the mild green strain and is in effect a part of the host cytoplasm Thus we have a well known virus from which we have separated one component which is related to the host and is not a unit in itself capable of independent expression That other yellow strains of viruses are of a similar nature is often suggested by their behavior in nature It remains to be ascertained whether other components of viruses may be separated in such a way that no virus remains even though the treatment has not been sufficiently drastic to destroy the respective components

It would be desirable to submit the viroplasm hypothesis to more intensive and extensive research than has yet been devoted to it Part of its interest lies in the failure of other hypotheses to explain satisfactorily the nature of a virus Certainly we should not need to seek an unknown kingdom of matter to explain the known facts

The Latin binomial system of nomenclature of plants and animals in general rests not so much on its own merits as on the fact that it is a convenient way of denoting classification When we use it for viruses, the nature of which is regarded as unknown we may further the confusion of classification of viruses

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THE RIO CONGRESS DECISIONS WITH REGARD TO STUDY OF SELECTED GROUPS OF VIRUSES

By C H Andrewes
National Institute for Medical Research London England

The fifth International Congress of Microbiology, held at Rio de Janeiro in August 1950 gave serious consideration to the question of virus nomenclature This was discussed by a section of the Congress devoted to Taxonomy by the International Committee on Nomenclature and its Virus Subcommittee and by its Judicial Commission These committees made various declarations and recommendations The decision which I am particularly to discuss in this paper was to designate five groups of better understood animal viruses for special study With them in the first instance, an attempt would be made to discover the applicability of certain principles to classification and perhaps provisional binomials would be suggested for them The viruses in question were the psittacosis lymphogranuloma group (Chlamydozoaceae), the insect pathogenic viruses, the pox group, the influenza group, and the arthropod borne encephalitis viruses For each group of viruses a convener was appointed with instructions to select one or two other workers with special experience in that field These small committees were to formulate proposals for treating their particular province of the virus field to get the views of other workers in that field to report to the main virus subcommittee and, eventually to the Sixth International Microbiological Congress to be held in Rome in 1953

You may well wonder why it was decided to attack the problem piecemeal and I must, therefore describe the background of this plan of attack Inquiries which I carried out before the Rio Congress made it clear that virologists were greatly divided as to the wisdom of applying to viruses binomial names in the Linnaean sense Questionnaires were sent to about 120 eminent virologists all over the world Replies were received from 46 plant virologists and 38 animal virologists from 23 countries The questions were worded so as to allow expression of all shades of opinion which was rather difficult to do in the case of the American Phytopathological Society s questionnaire Two out of three animal virologists thought that binomials should be applied at some time but only one in five wanted to do so immediately If left alone they would have done nothing about it Plant pathologists were very evenly divided as to whether binomials could be usefully applied to plant viruses or not They might have gone on indefinitely differing from each other peaceably or otherwise But Dr Holmes threw into the area a nomenclatorial bomb-shell which made serious discussion and if possible an agreed decision of a matter of urgency The position regarding animal viruses is this The large majority of experienced workers are to say the least, doubtful of the wisdom of applying binomials to viruses now, but 88 per cent are opposed to the suggested Holmes names Bergey s Manual however has a large circulation and some beginners in the virus field feel that they are in the fore front of progress if they use the names in the latest edition They do not

know that more experienced workers regard the Holmes classification as disastrous Such a situation inevitably leads to serious confusion It is therefore incumbent on all of us even those who are not convinced that binomial nomenclature is good to attempt to deal with this problem in an orderly manner A *laisser faire* attitude was well enough for plants and animals during the evolution of the principles of taxonomy and nomenclature but it is not good enough where it is a question of suddenly inventing a codified system *for all the viruses agents of interest to so many*

Here came the difficulty however The large majority of animal virologists will not accept Holmes s names nor would they I imagine accept at this moment any other set of names which might be propounded by any individual On the other hand a few are pressing for binomials for at least some animal viruses and what is more important the plant virologists are determined to have some sort of orderly nomenclature in their field It would seem advisable to avoid at all costs having the plant and animal viruses treated in diametrically opposite ways A compromise seems difficult to reach Dr Black chairman of the plant virus section of the International Virus Subcommittee finds his members in such violent disagreement that he treats them as two separate units a pro- and an anti binomial group each of which presumably meets separately and comes to unanimous decisions This seems to me a policy of despair for it is unlikely that any unanimous decision thus reached will commend itself to plant pathologists in general

I maintain that animal virologists are superior to their botanical colleagues in possessing a greater genius for compromise If we cannot agree to name all viruses or to name no viruses why not meet each other half way and name some viruses? This is what our subcommittee proposed at Rio I should mention here that with the exception of Dr Bitancourt no plant virologists were present at Rio and able to take part in these discussions A majority of those who answered my questionnaire was opposed to a piecemeal attack Presumably however many would prefer such a solution before a surrender to those holding wholly opposite views

The piecemeal attack has certain advantages The subject is so vast so difficult and in many parts so poorly understood that no one man could possibly cover it adequately nor could panels of experts survey the field within a short period Yet indefinite postponement of decision seems undesirable Certain groups of viruses are much better understood than others I refer especially to the five groups of animal viruses already mentioned Each is a fairly compact well-defined group There is hope that each small study team will be able to decide on what viruses to include how to apply to them the principles of classification how to subdivide them into genera and what names to bestow on such genera and the species therein I think that at present there can be no sense in trying to classify all animal viruses into families or into any taxonomic group higher in rank than the genus We do not know enough There will be time enough for that when a start has been made with lesser groups

May I indicate how I feel that we should deal with these virus groups?

First note should be taken *of the eight principles of classification suggested* by our subcommittee at Rio These will be of varying importance in different groups Thus morphology resistance to physical insults, tropism for epithelial tissues, and aptitude for forming inclusion bodies are properties of special interest in the pox group On the other hand, size natural routes of transmission and immunological properties will be particularly important among the encephalitis viruses Experiences of the different study teams will show which properties are of most general use In the discussions at Rio it was felt that one of Holmes s mistakes was to base far too much on tissue tropisms and symptomatology Knowledge of the lability of these properties (yellow fever is a good example) should show that their value is very limited I gather that many plant pathologists feel equally strongly that their importance has been magnified out of all reason in Holmes s classification of plant viruses.

Then the study groups will have to consider naming the inmates of their menageries Here they can feel free to accept or reject any name whether previously proposed by Dr Holmes or anyone else The Rio Congress, in *plenary session* unanimously passed a resolution that consideration of *the starting date for scientific nomenclature of viruses should be deferred to the* next Congress Most of the virologists whom I circularized felt that a valid starting date should be some date to be decided in the future (42 *vs* 29) There was no difference of opinion here between plant and animal virologists It is perfectly feasible for the study groups to propose names provisionally and for the sixth or any subsequent International Congress to validate these or other names later on as from a fixed date Until then they would have no status in nomenclature

It may be urged that names should be put forward by individuals as is normally done in other fields There are however precedents for group action of such a kind If a fixed future date for validating names is not decided upon, we shall forever be tied regarding priority of names for most animal viruses to Holmes s names I have no quarrel with Holmes's specific *names as names provided they are recognizably applicable to particular viruses* If they are not the study groups could get matters right by re-describing the species under the same name I do feel however that most of his genera bear so little relation to natural groupings that it would be far better to forget about them Their descriptions are in any case so sketchy and tenuous as to be positively immodest

I shall discuss the separate groups very briefly, as other papers will deal with them in this monograph

Psittacosis Lymphogranuloma Group (Chlamydozoaceae) To be dealt with by *Dr Rake this forms quite a natural group, lying as do the rickettsiae* between the bacteria and the more typical viruses It is a matter of great difficulty upon which agreement will be hard to achieve to decide whether to draw the bacterium virus boundary between the bacteria and the rickettsiae between the rickettsiae and the psittacosis group or between these last and the typical viruses I suggest that it might be the best plan to group and name the viruses concerned but to leave open for the present the question

of their whereabouts in the scheme of things It is perhap not a valid argu ment but keeping them with the true viruse would have the great advantage of permitting more freedom as to nomenclature and freedom to drop the abomination *Miyagawane 'a lymphogranulomatis*

The Pox Group This is again a fairly natural group—Sir Macfarlane Bur ret s particular care in the Rio plan—though there may be difficulties about placing *Varicella* and *Zoster* Goodpasture s generic name *Borreliota* seems omething definite to start from

The Influenza Group including Mumps Fowl plague Newcastle Disease and Influenzas A B and probably C seems very homogeneous to those study ing hemagglutination Recent discovery of hemagglutination among neuro tropic viruses may make the group harder to define From a clinician s point of view the infections concerned do not look at all alike (Holmes scatters the members among three genera) Nevertheless if we look at size stability and other fundamental properties as well as relations to red cell surfaces I think we shall find that the group is a natural one

The Insect Pathogenic Viruses If only on morphological grounds these seem to stand apart from others There is no risk of confusion with insect transmitted viruses of vertebrates or plants Dr Steinhaus seems very wise in uggesting that some knowledge of morphology should be insisted upon before new names are p oposed though I differ from him in not regarding all Holmes s names as being worthy of preservation in the interests of stability

The Arthropod Borne Encephalitis Viruses The e are perhaps dealt with le s readily than the other four groups They have to be differentiated on the one hand from the mouse encephalitis viruses of the FA and similar types and from the E M C (encephalomyocarditis) group The Mengo virus of this group seems to have been insect borne On the other hand they show many similarities in physical properties to the viruses of yellow fever and lymphocytic choriomeningitis

At present I see only two ways in which animal and plant pathologists can be induced to adopt a common line of action One plan is to agree that binomial nomenclature is at present unsuitable for any viruses The other is for all concerned to build on foundations of a consistency rather nearer to that of rock than of sand Some of us in the field of animal viruses are starting with agents of whose properties we do know a little If plant virologists adopt a similar course and begin to classify and name only the agents whose intrinsic nonpathological properties have been studied then we can march along together However to give the other plant viruses the imperfectly known viruses nonofficial binomials alongside the more official ones would in my view create terrible confusion

In view of the various other papers presented here I should like to emphasize that we may be discussing two quite different things in the field of virus nomen clature First there is binominal nomenclature of the Linnean type which falls under the rules of the International Microbiological Code of Nomenclature and the control of the International Nomenclature Committee and its Judicial

Commission Secondly, interested workers, for their own convenience, may decide on a numerical or other system for naming certain classes of viruses, but such arrangements are in a different class from those under the International Code Perhaps, the International Committee could be induced to give its blessing to such systems, but I do not think it has yet felt them to be within its province

ADVANTAGES OF THE LINNEAN BINOMIAL SYSTEM FOR PLANT VIRUSES

By P Limasset
Versailles France

Agreement on a system of virus nomenclature capable of ending the existing confusion has become absolutely necessary I shall not discuss the well known history of virus nomenclature The essential point is to examine the actual state of the question and to choose between the different solutions proposed today

For naming viruses some virologists favor the universal adoption of English name selected in a suitable manner Others prefer numerical systems like those presented by Johnson and K M Smith In these systems a virus is designated by the name of the host plant followed by a number The successive number correspond to the chronological order of the discovery of the different viruses Instead of using the whole name of the host plant K M Smith takes only the Latin name of its genus Some authors like Fawcett and Bennet, have proposed keeping K M Smith's principle of designating each virus by the Latin name of the host plant* followed by a specific Latin name replacing the number for example *Solanum vastans* Fawcett for potato-yellow-dwarf virus and *Nicotiana virus aclaphermus* Bennett for tobacco mosaic virus Finally many virologists would like to follow Holmes who first suggested a Latin Linnean system in which the virus species would be put together in genera according to their presumed affinities Holmes tried to apply his nomenclature to all viruses His generic divisions and his classification have been criticized but this should not cause prejudice to the principle of Linnean nomenclature

Personally I think that the first three systems mentioned (nomenclature using English terms numerical nomenclatures and derivations) have to be eliminated The use of English nomenclature does not seem well advised This principle is out of date since the denominations are based on symptoms now known to be of little specific value and on the names of host plants very often chosen in an arbitrary manner Moreover the names proposed in this system are really the names of the diseases and are applied to the pathogenic agent only secondarily by the addition of the term 'virus

The numerical systems have been very useful but besides the inconvenience due to the use of numbers they unfortunately have the disadvantage of imposing the rigid frame of an empirical classification Such a classification places different viruses together according to the series of host plants chosen in an arbitrary manner as in English nomenclature This system absolutely prevents the progressive establishment of a taxonomy based on the real affinities of the viruses K M Smith himself admitted that numerical nomenclature had become obsolete This system was adopted by many workers and

* The Latin [illegible] host [illegible] followed by th[illegible] [illegible] nomencl[illegible]

for a time it worked well enough However the continual discovery of new viruses not anticipated by the present writer at least has tended to make the system unwieldy and to create difficulties in remembering which number refers to which virus when many viruses are involved"*

Fawcett's Bennett's and similar nomenclatures have the same disadvantages since they *impose the same empirical classification* However they offer the advantage of eliminating numbers Moreover Bennett proposed his nomenclature as something temporary and suggested substituting a Linnean or a chemical nomenclature according to whether the viruses became recognized as living things or chemical compounds

Now we ought to consider Linnean nomenclature, which seems to have real advantages in comparison with the other systems

Definition and Advantages of Linnean Nomenclature This nomenclature used for naming all living things is characterized by the use of Latin and by a binary structure The first term of the binomial represents the genus the second the species

Linnean binomials became *prevalent during the eighteenth century*, before the discovery of the principles that have been used for the establishment of a taxonomy based on natural affinities Their flexibility facilitated all transformations of the classification The generic term applied to a unit small enough to avoid its disintegration, a result which would have produced very important changes in the terminology On the other hand it was wide enough to avoid the dismemberment of the specific denominations When the principle of the subordination of characters was established by Antoine Laurent de Jussieu it was easy to put together in families supposed to be natural the genera of higher plants scattered in the artificial Linnean system (these families were first called orders) Some modifications were made only in cases where the original formation of the genus was wrong Then divisions were made Some species were taken from the original genus and supplied a new generic appelation

This shows how unnecessary it is to wait to be able to classify most of the viruses according to their real affinities before applying Linnean nomenclature The bacteriologists undertood that perfectly, and they did not hesitate to use this nomenclature although the classifications proposed for bacteria are very questionable even today

The Linnean nomenclature seems to be as useful in virology as in botany and zoology It is the only one which permits placing the different viruses together according to their supposed affinities and leaving out little by little artificial or empirical classifications Latin is very convenient for that purpose having been established *by a long tradition, which it seems unwise to give* up Since it is a dead language, the terms used are for us very far from everyday life and can be adapted more easily to the designation of a new object without looking strange For example let us take the case of *Bacillus subtilis* The English translation of *subtilis* which is not funny in itself, is "subtle" or

* K M SMITH 19[illegible]1 Recent Advances in the Study of Plant Viruses 2nd ed. 288 Blakiston Philadelphia

sad which makes us laugh It would be easy to find similar examples showing that if living languages are well adapted to a nomenclature of common names devoid of any scientific character none of them would be suited to a Linnean nomenclature

Linnean nomenclature has many advantages It makes possible the grouping together of different viruses according to their affinities in the higher frames of classification It makes it possible to bring together all viruses having similar properties This synthetic work is useful even if many genera later appear to be heterogeneous and have to be divided which has happened many times in botany For example some genera of mosses are now considered as families or very nearly so Finally if the terms are carefully selected binomial nomenclature should help the memory not test it as the use of numbers does

The use of Linnean nomenclature however presents some objections These are of unequal value and we now have to examine the most important objections to see if the disadvantages do not outweigh the advantages

(1) *It is not certain that plant viruses or at least some of them are living things* If all plant viruses were living things Linnean nomenclature would have to be adopted without any discussion If we suppose however that the chemical nature of most of them will be established in the future the use of names simpler than those resulting from their very complicated structure will still be *necessary* In fact *this is now the case for vitamins and enzymes* Therefore we do not see why Latin binomials should be discarded for this reason Today they are used exclusively for naming living things but there are no reasons for not extending their use to viruses which have many similarities with living matter The knowledge of their chemical nature would only permit laying the basis of their systematics with a strictness and accuracy unknown today in biology

(2) *The concept of species and still more the concept of genus and family is difficult to establish in virology* This objection is the most serious one that can be made especially in regard to the generic concept which is the corner stone of the Linnean edifice We have to admit however that it is not peculiar to viruses although it is very important in this case The same difficulty although less marked has been found in bacteriology As soon as morphological characters become less important affinities are more difficult to determine Personally I think that this method can give satisfactory results in virology if it is wisely restricted to the best known viruses for example those which have been observed with the electron microscope and whose physicochemical properties have been determined with precision The principle of subordination of characters which is the basis of any good classification certainly is still valid for the virus scale This principle draws on the morphological criteria first Indeed I think that the form and dimensions of the particles have a very special importance They are certainly the visible expression of fundamental differences in structure The largest viruse are likely to have a membrane and an elementary internal structure This character separates them from smaller viruses which do not present such criteria Within this last category the round or elongate shape should indicate important

differences Therefore, I think that the morphological criteria are capable of providing a good basis for separating the natural families Among the well known plant viruses three categories can be distinguished viruses consisting of (1) very large particles (potato-yellow-dwarf virus), (2) small, elongated particles (tobacco-mosaic virus), (3) small round particles (tomato-bushy stunt virus) In each family the distinction between genera would be based on some secondary morphological details (small differences in the dimensions) and on physicochemical properties

One problem worth discussing is the importance that should be given to the mode of transmission In the last edition of his manual Bawden pointed out that from a taxonomic point of view, too great importance should not be attached to mechanical transmission because the factors that inhibit that transmission may be very variable in different cases It seems to be true however that viruses showing affinities are transmitted by vector insects belonging to the same zoological group (leafhoppers, aphids, thrips *etc*) This character should be taken into consideration at the family level or, within the *family, at a higher level than the genus* For definition of the genus *itself* other characters like the presence or absence of an incubation period or the effect of fasting would be better used in combination with the preceding criteria

Specific characters can be established on the basis of cross premunity and cross serological tests checked by the study of the most important physicochemical properties (temperature of inactivation length of storage without denaturation at 20 C filterability and crystallization) The mode of transmission also should be the same The criterion of premunity should be reciprocal to avoid the effects of nonreciprocal antagonism as it is furnished in one instance, by severe-etch virus and the potato virus Y As Kohler pointed out it should be noticed that if this criterion is valid when positive it would not be safe to draw final conclusions about the absence of affinities between two viruses when premunity tests are negative To be significant any study has to be checked many times and one can get very good evidence with premunity tests only when mechanical transmission is possible

When serological tests are possible they are of very great interest The different virus strains belonging to the same species must have a common antigenic group but each one may differ by its own small antigenic fractions When two viruses have a small antigenic group in common they should be considered as distinct species closely related especially if this conclusion is supported by other secondary characters * This is the case with tobacco-mosaic virus and the ribgrass mosaic virus (Holmes) The common antigenic group is reduced, and both viruses differ in their amino acid content and in the presence in the ribgrass virus of methione and histidine which are absent in tobacco mosaic virus

(3) *The properties of many viruses are not well known enough to make it possible to integrate them in a natural classification* It is true that this attempt at a natural taxonomy can be applied now only to very few viruses namely the ones observed with the electron microscope whose fundamental properties

* It should be pointed out th[illegible] serological [illegible] not be [illegible] for th genus bec[illegible] two closely rel[illegible] spec[illegible] reasonably h[illegible] [illegible] value

have been determined The number of well known viruses is increasing daily however and it now seems very necessary to build the framework of a classification where they can be placed one by one The less well known viruses can be given a temporary binomial and placed in a section apart clearly labeled *Imperfectly Known Viruses* Bawden proposed an analogous suggestion in the last edition of his book (page 276) The taxonomy of such viruses would be definitely artificial like the classification of the *Fungi imperfecti* In my opinion the Holmes nomenclature would be very well suited for that use It is based on symptoms and methods of transmission and is descriptive mnemonic and ready for use If necessary, it is possible to modify his system in some ways to lessen slightly its artificial character and as a result, to enable it to express some probable affinities

(4) *The form of some viruses could be an artifact produced during the extraction* It has been suggested that rod shaped viruses may be formed by the aggregation of round shaped elementary particles Some good electron microscope photographs made by Johnson permit us to postulate such elements even if they do not demonstrate their autonomy In that case the morphological criteria might be questioned I think however that even if we suppose that all the filamentous viruses are made of small round particles joined together (and this is far from sure) such an extraordinary mutual affinity of the particles is sufficient to make us think that it involves special properties The notion of a rod shaped virus would be changed to the notion of a linear colony The family of rod shaped viruses Baculaceae * would be defined in another manner but would keep its value At the very most a scission would be made if it was shown by further work that in some cases the rods were formed of elementary particles and in others by a chain of elementary round bodies Such scissions are common in taxonomy

(5) *Since there are not many criteria it will be difficult to imagine [illegible] binomials* This is a real difficulty but it does not seem in[illegible] Let us take the example of rod shaped viruses We can imagine the [illegible] Baculaceae with a type genus *Baculus* For naming other genera [illegible] *Bacillus* and *Bacterium* already used in bacteriology are excluded The name *Fustis* can be used to name viruses looking like *Baculus* [illegible] by some properties *Capillus* may designate very long and flex[illegible] but very soon it is impossible to find other words expressing the [illegible] We may then choose a name which reminds one of a symptom [illegible] this symptom is specific or very frequent As a name for a [illegible] also choose the name of a late virologist? In that manner [illegible] small arctic plant (*Linnaea borealis*) was dedicated to Linnaeus [illegible]
possible to use mythological names

The specific name may recall a very important host plant [illegible] for tobacco-mosaic virus) or a remarkable and frequent sym[illegible] *nervillustrans* for dahlia mosaic virus) In this case we can [illegible] clearing exists only in dahlia and is not constant In [illegible] for the symptom to be widespread and remarkabl For [illegible]

* All th names [illegible] examples [illegible] proposals
† Th gen [illegible] of th round shape [illegible] particle

cizes the name of *Geranium sanguineum* on the pretext that there are some white mutants, or the name of *Campanula rotundifolia* whose leaves are all elongated except for two or three round ones at the bottom that are difficult to see

Finally it is also possible to use names of localities Tomato-spotted wilt virus has been well named *Lethum australiense* by Holmes In the same way tobacco necrosis can be designated *Per uxor rothamstedi* * Authors' names can be used here also if employed with moderation

On the whole and in spite of real difficulties, I think that a minimum amount of imagination from the inventor, and a tolerance from those who only criticize should permit finding names for all the "imperfectly known viruses If not entirely satisfactory these would be practical and easy to use

Conclusion I hope I have shown that a Linnean nomenclature has very important advantages and that, by suppression of all synonyms and homonyms it permits progress, little by little, toward a natural classification Even if virus classification ceased to be biological and became entirely chemical there would be no reason for abandoning this Latin nomenclature It would be only something new in the history of science

I have discussed the objections which appear to me to be most important, to show that the disadvantages of Linnean nomenclature although real in some cases do not outweigh the advantages In the absence of a perfect solution it is wise to choose the best or, at least the best among the bad ones, and I am confident that the proposed system will appear excellent once it is in use

It seems to me that it is necessary now to build the framework of a natural classification in which it will at once be possible to place the best known viruses For the imperfectly known viruses I personally, think that Holmes's classification could be adopted as it is now or with some modifications

* The term *Per uxor* has been chosen to recall the old specific name *lethal* in Holmes nomenclature The specific name commemorates the important work done [illegible] the virus at Rothamsted

POSSIBILITIES OF GENERIC CLASSIFICATION IN RICKETTSIAE AND VIRUSES

By Geoffrey W. Rake
The Squibb Institute for Medical Research, New Brunswick, N. J.

I am no taxonomist. I know little of the formal laws and principles governing classification. It seems, therefore, foolhardy for me to attempt to defend the adoption of generic classification among the viruses and rickettsiae groups whose classification even into species is to be debated in this monograph. If the classification into species and families is difficult, that into genera is much more so. It is here that two distinct and often fiercely opposed varieties in the species *Homo sapiens* appear—the lumpers and the splitters. In no other part of taxonomy is the definition and delimitation so properly to be considered much as an art as a science, a matter of compromise between the two important criteria for arrangement into categories higher than species, namely, convenience and relationship.

There is no golden rule for the criteria of genera, even in zoology or botany. Since, in these fields, there exists the greatest possible divergence of opinion as to the proper delimitation of genera, how can I hope to satisfy more than a small proportion of my readers at this time? Julian Huxley has defined the problem of systematics as being that of detecting evolution at work. If you agree with me, as I must assume you do, that the evolution and therefore the systematics of viruses and rickettsiae do not differ essentially from those of animals or plants, then our problem is that of deciding, at our own particular moment in the flow of time, where the gaps have become sufficiently wide to allow the characterization of species but not too wide to allow inclusion within a genus. When the Linnaean system is used, the two names serve this very purpose, the species indicating width of gap sufficient to support specific definition, while the genus indicates a degree of similarity and thus presumed relationship such as to bind the species together. For this reason, perhaps above all others, it seems to me that if we agree that the time is ripe to use any systematic form of nomenclature, that form should be the Linnaean system. In that, I am in complete agreement with our Chairman. To me, nomenclature is useful only if it helps us to increase and bring together our knowledge of viruses and rickettsiae. Of all the proposed methods of nomenclature, the Linnaean system, when intelligently applied, is the only one which does so.

It has been pointed out above that generic classification is, in a large degree, an art. Within the genus must be placed such species as are presumably of common origin, while the separation of one genus from another depends upon the existence of a definite gap. It is clear that, particularly in early stages of knowledge, genera with only one species are possible, but with increasing knowledge the size of such a genus should grow. The reduction of genera to too small units with one or two species, as is the tendency of some classifists, is to destroy much of the value of the Linnaean system. In the higher animal, morphological similarity is one of the main criteria used for the segregation of

species into genera Even in zoological classification however while implied relationship is a prime criterion that of convenience i e, the use of classification as an aid to reference is only slightly less important in the selection of genera

Our Chairman has pointed out that a usable classification and nomenclature would be a great convenience to virologists particularly in going from one language to another Among the viruses and the rickettsiae, whatever their evolutionary story there appears to me to exist the same type of discontinuous continuity as occurs in higher animals presenting those degrees of difference sufficient for the selection of genera Where such degrees of similarity and difference do indeed exist among viruses and rickettsiae the convenience to be gained from undertaking now the preliminary steps in classification should entirely overcome the objection of imperfect knowledge Even in those fields in which classification and Linnaean nomenclature are firmly established and invaluable tools knowledge is still imperfect and will long continue to be so, but a start must be made somewhere

The importance of morphology in the classification of higher animals has been pointed out above In the smaller world of the viruses and rickettsiae with a few exceptions we do not yet have tools of sufficient delicacy or the full knowledge by which to make use of the tools we have to rely on morphological similarity for generic selection Other criteria must be considered Perhaps the most important of these are size serological character chemical and physical characters, tissue tropism, and invertebrate transmission in that order But this must be at present and for some time to come a matter for discussion

With all of the above in mind it seems clear to me not only that generic classification is possible both for rickettsiae and for viruses but that it is desirable We may argue as to whether the *Miyagawanellae* are rickettsiae or viruses (a point to which I shall return later in another paper) and we may deplore the clumsiness of the name in our Anglo-Saxon ears (as I do) But even the most carping critics have agreed that the agents of the psittacosis lymphogranuloma group are indeed "a group sufficiently separate and distinct to be set apart from other groups" In fact these agents present a degree of similarity in morphology, size, serological character chemical (tinctorial) and physical character, and tissue tropisms as to bind the different species together and separate the genus by a sufficient gap from other forms That there are transitional species whose exact classification is, in the light of our present knowledge uncertain or impossible should be a matter of no more embarrassment to us as virologists than the similar situation is to zoologists and botanists

Even among the agents agreed upon by all as being viruses it seems to me that we do not have to go beyond the *Borreliota*, the pox viruses to find a group of viruses with all the necessary criteria for generic classification As most of my readers will remember this genus was suggested by Goodpasture 20 years ago In my mind such classification was and is commendable and good

In my opinion the point seriously to be debated is whether we should admit

mono-specific genera I would believe that in some cases it may be desirable because of convenience and because of the presently unperfected state of our knowledge I am not however certain of *Homo sapiens* var splitter I believe that with such exceptions and they should be few two or more species within a genus are likely to serve what to my mind is the greater purpose of classification that of codification and thereby clarification of knowledge

In conclusion then I have set out certain criteria for generic classification and have pointed out that such classification is to a degree an art a compromise between convenience and scientifically demonstrated relationship I have indicated certain characteristics to be considered in the classification of viruses and rickettsiae which may be added to the largely morphological consideration of zoologists and botanists Finally I have indicated that when such criteria and characteristics are considered certain groups of rickettsiae and viruses present at this time all the features necessary for generic classification

CRITERIA FOR A BIOLOGICAL CLASSIFICATION OF BACTERIAL VIRUSES*

By Mark H Adams
New York University College of Medicine New York N Y

Since the application of the Linnean binomial nomenclature to living organisms is generally admitted to have been of value to the biological sciences it seems inevitable that this nomenclature will be extended to include the viruses The application of a binomial terminology however implies the existence of adequate criteria for defining the genus category and the species category At the present time there are no generally recognized criteria for determining whether two virus strains should be considered as two varieties of a single species or two species in the same genus, or two species in different genera We feel therefore that the use of trivial names for virus strains should be continued until an adequate experimental basis for a biological classification can be developed Until then, a purely practical name such as "coli-dysentery phage T5" should prove adequate to designate a particular strain of bacterial virus

For those interested in developing a biological classification as opposed to a purely practical determinative key, the following criteria for the biological classification of bacterial viruses are proposed These suggestions are based on the pioneering work of Burnet and on further information available in 1951 and are of course, subject to modification or elimination as the result of further laboratory work It is hoped that they may be a stimulus to further work clarifying the taxonomic relationships among the bacterial viruses

Serological Criteria

The partial or complete neutralization of the infectivity of one virus by the antiserum to a second virus indicates a fairly close biological relationship between the two viruses Absence of detectable neutralization however does not preclude a close biological relationship since circumstantial evidence indicates that serological specificity may alter through mutations The possibility of false conclusions must be recognized drawn from serological reactions due to contaminated reagents For instance the phage stocks used for antibody production may contain a second phage derived from a lysogenic host bacterium (carrying a latent virus) To be definitive the serological relationship *should be independent of the host bacterium on which the antigenic phage* stocks have been grown Complement fixation and agglutination reactions probably are less reliable than neutralization of infectivity because of the danger of contamination of the virus stocks with extraneous nonviral antigenic materials such as host cell antigens although this danger can be mitigated by absorption of the antiserum with host cell antigenic material

The value of serological relationship as an indicator of biological relationship was demonstrated by the extensive work of Burnet[1] with the coli dysentery and

* Aided by a grant from the National Foundation for Infantile Paralysis Inc

(4) Osmotic shock (Anderson[11])
(5) Sonic vibration (Anderson[1])
(6) Heat (Burnet[13])
(7) Low salt concentration (Adams[14])
(8) Inactivation on drying (Kohn[15])
(9) pH stability range (not yet applied to bacterial viruses)
(10) Surface inactivation (Adams[16])

None of these inactivation methods has been studied over a very extensive range of bacterial viruses so it is impossible to state at present, the relative importance of each to taxonomy All of them are easily studied however and only the sonic generator may not be generally available to research workers Further work will be required to decide which of these properties are most useful in virus classification

(*B*) *Specific Growth Requirements of the Virus* Burnet[9] showed that certain groups of closely related viruses shared a requirement for calcium ion The host cell and other unrelated viruses multiplying on the same host cell grew very well in the absence of calcium To the present this is the only nutritional requirement of viruses which has been extensively investigated but it seems to be a useful indicator of biological relationship The calcium requirement has not been conclusively demonstrated to alter by mutation

(*C*) *Multiplicity Reactivation of Ultraviolet Inactivated Bacteriophage Discovered by Luria* [10] When a susceptible host cell is infected with a single, ultra violet inactivated phage particle the host cell is killed but the virus does not multiply When a similar host cell is infected with two or more inactivated virus particles virus multiplication occurs in a proportion of multiply infected host cells which depends on the dosage of ultraviolet light used Multiplicity reactivation occurs with the members of the T2 T4 T6 serological group and with T5 and its serological relatives but not with the T3 T7 group or with T1 and its serological relatives so the phenomenon is correlated with the serological grouping of the coli phages Whether it occurs with other phages is not known

(*D*) *The Occurrence of Parallel Mutational Patterns* The only case of this kind that has been studied so far is the mutation from r+ to r studied by Hershey [17] This mutation occurs at a rapid rate in the members of the T2 T4 T6 serological group of coli phages but does not occur with the rest of the T coli phages It seems probable that other mutational patterns of diagnostic significance will be found

(*E*) *Other Physiological Properties Peculiar to Certain Groups of Phages* It seems probable that intensive research will uncover additional examples of biological properties of diagnostic significance The phenomena of lysis from without (Delbruck[18]) lysis inhibition (Doermann[19]) and host killing by ultra violet inactivated phage (Luria[10]) may be examples of such properties Only more extensive studies can tell how useful such properties may become to phage taxonomy

Because of the dearth of morphological characteristics in viruses it seems inevitable that physiological characteristics such as those enumerated above will be used in viral taxonomy as morphology has been used in the case of

higher organisms The criteria are comparable since hereditary morphological varieties are certainly the result of physiological variation Many of the physiological characteristics mentioned are subject to change by mutation and this interferes with the use of single properties as taxonomic criteria It is this *mutability* however that has generated the bewildering array of phage varieties which are known today Presumably the more closely related are two virus strains the fewer will be the differences in their physiological properties It is quite impossible at present to express such relationships in a quantitative manner More precise knowledge of the mutability of physiological properties will permit a choice of the more constant characteristics as taxonomic criteria

Results of Mixed Infection as a Taxonomic Criterion

The simultaneous infection of a single host cell with virus particles of two different strains each capable of multiplying in the host by itself may result in either of the following phenomena (a) mutual exclusion in which the mixedly infected host cell liberates one or the other infecting virus but not both and (b) mixed bursts in which the mixedly infected host cell liberates both infecting virus types and often new types resulting from recombination of genetic elements derived from both infecting virus strains

These phenomena have been investigated so far only with the T series of coli phages and their close relatives but the results have been clear cut and unequivocal

If the two infecting virus strains are unrelated by the serological criterion the result of mixed infection invariably has been mutual exclusion Of the 21 pairs of viruses that can be formed of the seven phages of the T system four involve pairs of serologically related viruses Of the 17 unrelated virus pairs all but two have been tested by mixed infection and all of these were found to demonstrate mutual exclusion [0-23] Mutual exclusion has also been demonstrated between phage T5 and the serologically unrelated prophage of the lysogenic strain K12 of *Escherichia coli* after ultraviolet activation [24]

In sharp contrast are the results of mixed infection experiments involving pairs of serologically related viruses Mixed infection with the serologically related virus pairs T2–T4 T2–T6 and T4–T6 resulted in mixed bursts and recombination of genetic characters [25, 26] Similar observations have been made with the virus pairs T5–PB[27] and T1 mutants [28] The T2 T4 T6 group is serologically unrelated to the T5–PB pair and neither is related to T1 Therefore within the membership of each of three distinct serological groups of coli phages (all that have been tested) mixed infection has resulted in mixed bursts and genetic recombination These observations must be extended before it is safe to make generalizations but it is indeed suggestive that Burnet[29] has reported genetic recombination following mixed infections with members of the serological group A of the influenza viruses If genetic recombination should prove to be a general phenomenon following mixed infection with pairs of closely related viruses it would be a finding of great importance to the taxonomy of viruses

A major problem in taxonomy has been the definition of species with the

(4) Osmotic shock (Anderson[11])
(5) Sonic vibration (Anderson[12])
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adequately characterized virus strains as types for each new species proposed It is hoped that some machinery for maintaining and distributing type cultures and type antisera will be set up before general adoption of a binomial nomenclature for viruses

It would seem more profitable in the immediate future to extent the techniques already worked out with the enteric phages to as many different kinds of bacterial viruses as possible rather than to attempt a classification of these organisms based only on present fragmentary knowledge Such extensive work might well uncover additional characteristics of greater taxonomic value than those suggested here

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development of criteria to distinguish species from races, varieties and other subspecific categories This problem has been exaggerated in the case of microorganisms because of the lack of any criterion for defining the limits of a species Such a criterion based on the occurrence of interbreeding has become widely accepted for sexually reproducing organisms Two groups of organisms that are physiologically incapable of interbreeding must be placed in different species whereas two groups of organisms that habitually interbreed or are potentially capable of interbreeding if geographical barriers are overcome, are included within the same species [20] This criterion is, of course not applicable to asexual organisms In the case of viruses however, mixed infection followed by genetic recombination is analogous to interbreeding in higher organisms since both are methods for the interchange of genes Therefore the occurrence of genetic recombination between the two virus strains may be taken as evidence that they are genetically compatible and should be included in the same species Conversely the occurrence of mutual exclusion is analogous to physiological sexual isolation in higher organisms and would indicate that the virus strains concerned should be placed in different species The application of this criterion to viruses is dependent on the availability of a suitable common host strain for the mixed infection experiments The failure to find a common host for two virus strains would be analogous to geographical isolation among higher organisms and would indicate nothing with respect to a possible relationship of the two virus strains By these criteria phage strains T2 T4 and T6 would be varieties in one species while strains T5 and PB would be varieties in a second species

Not all criteria listed above would be applicable to all pairs of viruses to be tested for possible relationship Therefore it is tentatively suggested that similar conclusions drawn from criteria I and II I and III or I and IV or from any three categories, would constitute adequate evidence for placing two virus strains in the same species or for excluding one of them from the species

The adoption of a binomial nomenclature involves the recognition not only of the species category but also of the genus There seems to be no natural way of defining the genus category among bacterial viruses One possible solution proposed by Holmes [21] is to lump all species of bacteriophage into one genus This suggestion defeats one of the principal aims of classification that of conveying information through the choice of categories It also creates a rather unwieldy genus containing in the proposal of Holmes 40 species A possible criterion for the genus category is size and morphology in the electron microscope This valuable research tool has been rather neglected by phage workers but from what is already known the phages vary enormously in size and shape from one strain to another A more extensive survey of morphology among known strains of phage should indicate whether this criterion is applicable to the definition of the genus category In any case it is more desirable to postpone the adoption of a binomial nomenclature for viruses until such time as some generally acceptable definition of the genus category may be proposed

The application of these criteria to phage taxonomy will depend on setting up

Bawden (1939 1943) was probably the first to call the attention to the similarity of the situation of the imperfectly known viruses and that of the imperfect fungi He remarked that a classification of fungi on systematic lines was attempted long before all the members could be placed in one or the other of the main groups Those insufficiently known were placed in a group apart and clearly labeled *Fungi imperfecti* He concluded that there seems to be no good reason why viruses should not be treated similarly and proposed that the viruses that are not transmitted mechanically be conveniently put in a group together They should have a name chosen to show that this is the reason for putting them there and not that their properties are sufficiently known to be certain that they are closely related

In his latest discussion of this subject Bawden (1950) again stresses that many viruses will remain unclassifiable for a long time and to avoid a spurious air of knowledge with its possible repercussions on research we should classify only those about which we have a reasonable amount of information The rest should be set apart provided with names suitably chosen to stress our ignorance of their properties and they should remain *in limbo* until there is enough knowledge to justify their removal

Valleau (1940) recommended that the viruses that appear to be found on only a single host for which relationships are not known and for which there are no well defined characters be placed in some catch all genus In this class he placed the majority of viruses in Holmes s genus *Marmor* and many of those listed in Holmes s supplement III already cited He suggested that inasmuch as Holmes made no attempt to define the genus *Marmor* it might be defined as including viruses causing diseases usually characterized by persistent mottling or necrotic spotting on some genotypes and which have not been sufficiently studied so that their relationships to other recognized viruses are known Valleau later suggested (1941) that more than one genus could be established for those little known viruses and remarked that the group he proposed is comparable to the very useful *Fungi imperfecti* of the fungi

As we have already seen McKinney (1944) recognized that the six genera of his classification that have been transmitted only by tissue union or by prolonged contact of tissues may have to be transferred to appropriate genera or to new genera as their vectors are discovered or as mechanical transmission is effected He stated that the six viruses that fall in this category fulfill the purposes of the single temporary group suggested by Valleau (1940)

Quite recently Limasset (1948) espoused the ideas of Valleau and Bawden He suggested that imperfectly known viruses be grouped provisionally in an artificial family he called the Invectoriae comparable to the imperfect fungi an artificial grouping of fungi the sexual stage of which is unknown Such viruses could not be legitimately included in a great natural family for this would suggest relationships that would later be likely to reveal themselves as inaccurate In the *Invectoriae* he proposed that the genera be distinguished *on the basis of symptoms induced on the host as for example the* genus *Pseudomarmor* for those that cause mosaics *Chlorophthora* for the yellows and *Rimocortius* when there is a shelling of the bark of the host He would also place in this group such viruses as *Nanus sacchari* the hypothetical cause

DISCUSSION OF THE PROBLEM OF IMPERFECTLY KNOWN VIRUSES*

By A A Bitancourt†
Instituto Biol gico São Paulo Brasil

This discussion was prepared with plant viruses in mind It is hoped that, to a great extent, it will apply also to animal and bacterial viruses

It is generally recognized that only an extremely small number of viruses are sufficiently known to furnish the bases of a natural classification In most of the schemes that have been proposed the lesser known viruses have been ignored or have been set apart from the main classification Johnson and Hoggan (1935) have kept out of their classification 69 plant viruses on which sufficient information was not yet available to allow them to be definitely placed in their key They listed a large percentage of those known at that time in such a way as to indicate some of the chief diagnostic features that were lacking

Holmes (1939) has listed in supplement III of his handbook 51 viruses about which little was known and for which he did not provide a Latin name In his last and more ambitious treatment (1948), he grouped 40 species in the genus *Marmor* in what he called a Miscellaneous Mosaic Virus Group These species stand in need of reinvestigation to determine additional properties and possible relationships to preceding groups of *Marmor*

McKinney (1944) limited himself to the description of the type species of the 18 genera of his classification However he places six of them that have been transmitted only by tissue union or by prolonged contact of tissues and which therefore have unknown intrinsic properties in each host reaction group in which they occur He suggested that those viruses may be transferred to appropriate genera or new genera may be established as the vectors are discovered or as transmission by expressed juice is effected

Other authors like E M Johnson (1930) Quanjer (1931) and Valleau (1940) have restricted their treatment to the systematic arrangement of only those viruses with which they were familiar

In spite of this cautious attitude the fact is that in many instances insufficiently known viruses have been included in these schemes thus suggesting relationships that may later prove nonexistent, when the advance of knowledge will provide better characters for their classification For instance Holmes (1939) has placed in the genus *Marmor* the virus of Abutilon mosaic Even though he later (1948) removed the species together with several other ones to a separate group of miscellaneous mosaic viruses it still remains in that genus which contains mostly viruses transmitted by aphids The discovery that the same or a closely related disease is transmitted by white flies (Orlando and Silberschmidt 1945) casts some doubt on the appropriateness of this classification

* In the program f th onf ence wh h th m ograph b ed th xpres *V a mperfecta* w sed S ce th L t V s I pt a d I d l ble th s t rm is in dm sable. Th Engl h phrase Imperfectly Know V ruses s th ref re b t t t d in th t t wh e r it req red

† It s plea to acknowledge th h lpf l comm ts of Drs L M Bla k K. Silberschm dt M Kramer d M M gh ni wh h kindly re d th m uscr pt

among them Furthermore this fact does not imply that a classification based *on the characters of the imperfect stage may not eventually lead to a natural* or phylogenetic arrangement of its members It has been pointed out by several authors that frequently there is a similarity in asexual structures among fungi considered to be closely related as judged by their perfect stage (Bessey 1950) In fact it is probable that in all cases in which fungi from different families or orders of the sexual stage have been described in the same form genus the mycologists have been misled by superficial resemblance The system of the *Fungi imperfecti* is thus amenable to improvement and a better knowledge of the asexual fructifications may lead eventually to a natural system parallel to that of the sexual stage

Even if this were not so mycologists could not do without it because of its great practical value There are now probably well over 20 000 species of the *Fungi imperfecti* which as far back as 1931 were already divided into 1331 form genera (Bessey 1950) Inasmuch as a great many fungi are known so *far in their imperfect stages only or are found most of the time in nature in* asexual forms of fructifications the mycologist confronted with the necessity of identifying a newly found fungus would be entirely at a loss if he did not have the help of the practical system of classification of the *Fungi imperfecti* Nor can this system be considered as a provisional one the asexual stage ceasing to be known by its name among the imperfect fungi and no longer included in that group once the perfect stage has been discovered In fact for practical reasons it is desirable to retain this name among the *Fungi imperfecti* since it would be sought there in attempts to identify it unless the perfect stage were found along with it (Bessey 1950)

Returning to the viruses we can now draw a parallel of the situation of the imperfectly known ones and that of the *Fungi imperfecti* There is no such thing as an imperfect stage in the viruses although the possibility of crosses and recombinations of characters in the bacterial viruses (Hershey and Rotman *1949) points to some sort of primitive form of sexual reproduction which might* some day serve as the basis for a natural classification of the viruses While the name *Fungi imperfecti* however refers to the fact that their classification is based on the characters of the imperfect stages the truth is that because in many cases the perfect stage actually exists but has not yet been found the connotation has become that of imperfectly known entities

Several authors have suggested that the classification of the Imperfectly Known Viruses be treated in a way similar to that of the *Fungi imperfecti* This seems well warranted

What should be the basis of the classification of the imperfectly known viruses? The aim being that of a practical system with the main purpose of enabling the identification of newly found viruse most of the criticisms of the existing systems based on symptomatology are not valid here Besides there is little else on which to found the classification because by definition little is known of the intrinsic properties of these viruses especially if the group is to contain mainly those that have not been transmitted by other means than grafting or prolonged tissue contact Admittedly such a classification will not show true relationships It has been rightly observed that a classification

of sereh of the sugar cane, and other viruses of which the existence is still uncertain.

Except for McKinney, who placed the imperfectly known viruses along with the better known ones in each host reaction group in which they occur, all of those authors have thus stressed the necessity of setting apart this considerable group of viruses. It is quite appropriate, therefore, that a place for the treatment of the imperfectly known viruses should have been included in this monograph. Inasmuch as this has been inspired by the similar situation obtaining in the fungi, it seems worthwhile to start this paper with a very brief description of the classification of the fungi.

As pointedly stated by Bawden (1950), the taxonomy of fungi was attempted long before all the members could be assigned to genera or families, the uncertain ones being placed in a section apart and clearly labelled *Fungi imperfecti*. With the progress of mycology and the recognition of a sexual form of reproduction in all the known divisions, it became plain that, just as in other groups of the plant and animal kingdoms, the best characters for the establishment of a phylogenetic classification were those of the sexual stage, also sometimes called the perfect stage. A great many fungi, however, are known only in asexual forms of reproduction or fructifications, the so-called imperfect stages or imperfect forms. The older mycologists were not aware of the distinction between the sexual and asexual stages, and named many fungi after their imperfect forms. In order to facilitate their classification, it has been necessary to group them in a systematic arrangement based on the morphology of their fructifications. This is not a natural or phylogenetic classification. It is essentially a practical system which, incidentally, shows that, contrary to the assumption of some of the discussions on the taxonomy of viruses, a classification does not necessarily have to be a natural one to serve a useful purpose.

Under such a scheme, the genera are no longer natural groups, and the mycologists have coined the name "form genera" to distinguish them from the natural genera of phylogenetic classifications. The same fungus may thus have several names, one for each of its asexual fructifications and one for the sexual stage. The fungus that causes the rust of *Soldanella*, with its four names *Aecidiolum soldanellae*, *Aecidium soldanellae*, *Uredo soldanellae*, and *Puccinia soldanellae*, one for each kind of its fructifications, is an extreme case of this form synonymy, which must be distinguished from pure synonymy.

Due to the artificiality of the classification of the *Fungi imperfecti*, one form genus may often contain species pertaining to different families or orders in the natural classification of the sexual stage. The genus *Gloeosporium*, for example, has species from different orders of the Ascomycetes, such as the Sphaeriales, Pezizales, and Myriangiales. On the other hand, species of the same perfect genus, *Elsinoë*, for instance, have asexual fructifications which, owing to differences in structure, have been assigned to form genera in the Moniliaceae, Tuberculariaceae, and Melanconiaceae of the *Fungi imperfecti*.

In spite of those shortcomings, no mycologist would ever think of eliminating the *Fungi imperfecti* from the classification of fungi, because it is well understood that it is a practical classification, the main purpose of which is to assist in the identification of fungi, not that of showing true relationships.

new names for genera showing some resemblance to or derived from older genera by adding suffixes like *oides odes opsis ella ina* to the older name In the case of the form genus *Sphaeropsis* for instance the name indicates a similarity of structure of its asexual fructification a pycnidium with that of the sexual stage or perithecium of the extinct genus *Sphaeria* The order Sphaeropsidales and the families Sphaeroidaceae and Nectroidaceae have been named according to a similar criterion More often than not however the suffixes have been applied to perfect forms and merely connote the resemblance

In the case of the imperfectly known viruses however there is no reason why a convenient suffix might not be chosen to indicate that the genus is provisional * and applies to little known viruses that cannot be included in one of the genera of the natural classification This at least seems a possible way of fulfilling the recommendations of Bawden (1950) that the names of the imperfectly known viruses be suitably chosen to stress our ignorance of their properties Thus instead of *Marmor* or *Pseudomarmor* as suggested by Valleau and Limasset respectively a virus inducing mosaiclike symptoms would be called *Marmorodes* and would be included together with the genera *Aerogenodes Coriodes Nanodes Rimocortiodes* and *Adelanodes* in the family Marmoropsidaceae Other families would be Chlorogenopsidaceae Annulopsidaceae Rugopsidaceae Savoiopsidaceae and Lethopsidaceae Obviously many of the genera from which the imperfect genera would thus be derived will for a long time remain without species and will be put aside for the time being These viruses will be returned to the natural classification if enough becomes known about them to put them back there on firmer grounds than they were originally

The flexibility of such a nomenclature is obvious Its provisional nature will be expressed by the suffix and there will be little necessity of changing names until the species is put into the natural classification

Something should now be said about the authorities for the virus names in the imperfectly known viruses and in this connection Johnson s apt remarks (1949) deserve reproduction here The authority given for the virus name is a matter worthy of careful consideration Under the Latin Binomial System the final authority is traditionally the person who applies the new name At the present stage of virology this may impose a considerable handicap to both the old and the new students of the subject The inclusion of the authority for the first reliable description of the virus in question regardless of the nomenclature applied *would not be burdensome It would add greatly to quick* recognition of the identity of the virus even if the Latin name offers little in this direction the exact form of the technical name of a virus under the International System of Virus Nomenclature (ISVN) awaits final decision by the Virus Committee of the International Botanical Congress

Johnson adds The authority for the first adequate description is to be shown followed by the authority for the name of the virus such as ISVN

In compliance with such a recommendation the insufficiently known virus

* F wcett (1940) h ggested ystem f m lature i wh h *p temp* g era re amed afte th host f th first desc p f llowed by th uffi es as Cit ir f an Th y em w t intended t be ppl ed t y l as ficatio f th ruses d i f t does t l d ts lf to ch an pplicatio

based on symptoms is in fact, a classification of diseases, not of their agents and that symptoms are eminently variable, influenced to a remarkable degree by the variety and growth stage of the host at the time of infection, the environmental conditions and other circumstances (Johnson, 1949) Yet if those factors are duly taken into consideration symptoms very often will define a virus quite well The reactions the bacteria induce in suitable substrates are extremely varied Nevertheless they are used for the determination of many genera and species because it is always easy to specify clearly the nature and condition of the substrate Symptoms are the reactions induced by the virus in the living substrate of the host There is no reason why, with better knowledge of the hosts' reactions at the microscopical as well as the macroscopical level the proper choice of symptoms on differential hosts inoculated at a given stage of growth, will not serve as adequate criteria allowing for an unambiguous characterization of viruses Just as in the case of the *Fungi imperfecti* the classification of the imperfectly known viruses although mostly based on symptoms will be perfectible and in many cases will indicate natural relationships But again as in the *Fungi imperfecti* this is not the most important requisite or purpose of such a classification

It would therefore seem that a classification such as Holmes s scheme in its latest form (1948) might well serve as the basis for the creation of a separate group of the imperfectly known viruses with a convenient nomenclature, as will be explained later Thus there would be, in the beginning two almost parallel classifications, one for the best known viruses and another one for the little known ones but this parallelism will soon be lost as the progress of our knowledge will cause rapid changes in the natural classification of the well-known viruses and much slower ones in the imperfectly known viruses

What viruses should be included in this category? Obviously the majority of the viruses known today and especially those of which the only known mode of transmission is grafting or prolonged tissue contact should be included *They will be excluded from natural classifications due to the lack of knowledge* of their intrinsic properties the indispensable characters in schemes aiming at the establishment of true relationships between their members They will thus be separated from closely similar better known viruses already placed in the natural classification but this inconvenience will be minimized by an adequate nomenclature as we shall see presently

On the other hand, as recommended by Fawcett (1940) no suspected virus should be given a name merely from observation of certain symptoms or effects in nature that resemble virus diseases until it had been conclusively proved that it is transmissible from plant to plant by budding grafting use of insect vectors mechanical or other means It would serve no useful purpose to include even among the imperfectly known viruses hypothetical entities such as the suspected agent of the sereh disease of sugar cane (Limasset 1948)

We are now ready to consider the question of nomenclature There are no provisions or recommendations in the International Rules of Botanical Nomenclature (Briquet 1935) to differentiate the names of form genera from those of the perfect genera and this is regrettable Mycologists have often established

VIRUS AND RICKETTSIAL CLASSIFICATION AND NOMENCLATURE

By Herald R Cox

Lederle Laboratories D s on American Cyanamid Compan Pearl River N Y

Nordenskiold in his History of Biology remarks that As long as man s knowledge of nature is limited to what he can observe in his immediate vicinity he has little difficulty in controlling the objects of his knowledge but when his range of vision widens there arises the irresistible need for combining the in dividual object that have been observed under general expressions which serve to fix the knowledge of them and to impart it to others

The conference on which this monograph is based was called because col lectively we felt that the time had come when we needed to survey the agents with which we work and to canvass the knowledge and opinions of the group in order to determine whether or not we are working with the same things and meaning the same agents when we use certain terms By grouping related things together we are certain to arrive at a better understanding of them

One of the distinguishing characteristics of the scientist is his desire and need to classify Classification however need not be ritualistic Its only justification is usefulness It is not holy writ but merely an intelligent guide to the good life scientifically We should not lose sight of the fact although it is commonly done that systems of classification are created by man not by God and subsequently are subject to all the faults and inconsistencies that will always attend man s creative efforts Nature is not a system of neatly arranged pigeon holes but is rather a series of continuous spectra in which one group shades and blends at the borders with its adjacent neighbors

In the rickettsiae it appears that a rather solid foundation already exists upon which to build further subject of course to various alterations that will be necessitated from time to time as our knowledge concerning the basic properties of these agents increases I believe that the eight basic criteria suggested by the virus nomenclature committee at the Rio conferen e and more recently cited by Andrewes in his lecture on Viruses and Linnaeus apply just as well to the classification of rickettsiae as they do to viruses Here I wish to pause a moment to pay tribute to one of the eminent scientist of our time a man who unfortunately has been given too little credit at least in this country This man is Dr Da Rocha Lima the distinguished Brazilian scientist who named the causative agent of typhus fever *Rickettsia prowazekii* after the American and Austrian scientists who died of typhus fever while studying its etiology It was Dr Da Rocha Lima who also made the much more important contribution of conclusively proving the morphological and etiological identity of the causative agent of typhus fever

As Steinhaus has stated *Rickettsia prowazekii* is the geno type species and it has been so accepted by all workers in the field It forms the basic nucleus about which discussion of the group in general and of the genus *Rickettsia* specifically should be centered

of the infectious mottling of *Citrus*, for instance, first described by Petri in 1931, should be named *Marmorodes italicum* (Petri) ISVN if approved by the Virus Committee of the I B C

I hope that the virologists who might eventually be tempted to establish a complete scheme for the classification and nomenclature of the imperfectly known viruses along the lines which have just been roughly traced or otherwise will follow Johnson s recommendations They should refrain from publishing their complete system in final form but instead they should unselfishly submit it for the approval of the International Committee In the event that this committee approves such as classification, it will be considered as the final authority for the name of the viruses thus described

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CLASSIFICATION OF RICKETTSIAE PATHOGENIC TO VERTEBRATES

By J D W A Coles

Division of Veterinary Services Department of Agriculture Onderstepoort South Africa

At the commencement of this paper it is necessary to present my credentials so that you may know how much importance to attach to my statements Obviously no one person can be an undisputed authority on all aspects of all the Rickettsioses and it would be idle to pretend that some at least of my beliefs will not be challenged

My interest in the rickettsias during the past 20 years has amounted to no more than a hobby owing to my preoccupation with other duties I discovered the rickettsias associated with and probably responsible for conjunctivitis and keratitis of the sheep ox goat and fowl I found the very large and apparently harmless rickettsialike organisms occurring often in the conjunctival epithelial cells of the sheep ox and goat I was able to show that the conjunctival inclusions reported by Uhlenhuth and Boing in cases of hog cholera had nothing to do with this disease but were rickettsias probably causing an independent conjunctivitis I also described the first cases of psittacosis or ornithosis in pigeons

I think that I have a reasonably sound knowledge of *Rickettsia ruminantium R canis R bovis R ovina* the organisms found in the monocytes of pigeons by Canham and the inclusions of trachoma and swimming bath conjunctivitis I have seen many cases of South African tick bite fever of man and know a little about *Rickettsia pijperi* assuming this species to be valid Some years ago in Oregon I was fortunate enough to be able to examine dogs suffering from salmon poisoning and recently I have studied a few excellent smears and sections containing the rickettsias furnished by Cordy I have only a superficial knowledge of *Coxiella burnetii Rickettsia prowazekii R typhi R rickettsii* the rickettsialike organisms associated with tick borne fever of sheep and the etiological agent of enzootic abortion of ewes

Having made these claims I must hasten to confess that I have done no serological work on any of the rickettsias and have relied almost entirely on Giemsa as a stain Rightly or wrongly I feel that no stain can equal a good brand of Giemsa for revealing all the details of morphology By now you must be painfully aware of my defects Perhaps the only saving grace is that I have not studied or worked in a water tight compartment but have taken an interest in many representative individuals of that large and fascinating community of very small and very parasitic parasites known as the rickettsias

The Nature of Rickettsias

It is surely unnecessary to argue that these structures are not reaction bodies produced by viruses The most rational conception of them seems to be that they are very pleomorphic and highly specialized bacteria that multiply in or in intimate association with living body cells that are not erythro

Concerning the nomenclature of viruses I find myself in agreement with Andrewes in that we must give full credit to Holmes for at least having stirred the animal virologists to take an interest in virus taxonomy, even though we do not agree with his classification The next ten papers and those which follow in contrast to the preceding ten papers are devoted to discussion of the classification of specific groups of viral and rickettsial agents I should like to remind the contributors that Linnaeus in attempting to classify *Bacteria* in his Systema Naturae ' renounced the task and apparently with an outburst of temper assigned these minute organisms to the class Vermes to which he gave the name 'Chaos *Choas infusoriorum* I hope that the final results of these papers will not be Chaos *Chaos virus*

Many of the properties that have been so useful to the botanists and zoologists are quite beyond us, and we must seek new and different bases appropriate to the agents we are attempting to classify It matters less what we call this or that virus or group of viruses than what we elect to accept as the basic properties by which these individuals and groups are to be recognized

Regardless of what scheme or system we ultimately adopt we shall, in all likelihood go on calling viruses in our daily work by the same names we have used in the past, but in our recording and reporting some names with recognized meat and meaning can certainly help to dispel and avoid some of the mounting confusion in the most intensively worked fields of research

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stain purplish red with Giemsa though many of the smallest bacillary forms are blue

The exact color of rickettsias in a colony is not always easily discernible if there is a fairly compact matrix This trouble arises in the case of *R. canis* for example where the matrix is acidophilic and usually dense and purple The scanty basophilic matrix in psittacosis presents no difficulties Neither does the glycogen containing matrix of the trachoma inclusion so long as it is stained with Giemsa The nature of the matrix may be of assistance in classify ing rickettsias but at present operates only in the case of the genus *Chlamy do oon*

Rickettsias occur in more or less well-defined colonies in which the granules may be alike or even greatly dissimilar In some species as many as 10 to 20 colonies may be seen in one cell The position assumed by a colony in a cell may assist in the process of identification In trachoma and inclusion blen norrhoea the colonies cap the nuclei of the conjunctival epithelial cells In heartwater they adjoin the poles of the nuclei in the elongated endothelial cells of the capillaries In the conjunctival epithelial cells of fowls the rickett sias tend to congregate round the periphery but whether they prefer the mar ginal zone of a cell or are repelled by its nucleus is unknown Colonies of *R bo is* and *R canis* often push so hard against the nucleus that they lie in marked depressions on the surface When situated like this the colonies are reminis cent of the gametocytes of Leucocytozoa snuggling into the nuclei of immature leucocytes

We have already given some indication that the kind of cell parasitized is of considerable diagnostic value To what has been said we may add that *Miyagawanella psittaci* has a predilection for foam cells just as *Rickettsia canis R bovis R ovina* and Canham s organism have for monocytes and the causal agent of salmon poisoning for reticulo-endothelial cells of the lymph glands tonsils spleen and thymus

It is most risky to attempt the classification of rickettsias by examining only a few preparations The uninitiated could be baffled completely by being asked to report on half a-dozen smears from as many sheep suffering from heart water Probably pleomorphism is manifested to the greatest extent by the rickettsias responsible for heartwater psittacosis and conjunctivitis of sheep and least by the members of the genus *Rickettsia*

Though I am convinced that granules of one type may give rise to granules of other types I have never been able to satisfy myself that anything like a definite life cycle exists

Furthermore I believe that rickettsias never occur in the guise of large homogeneous plaques These plaques are very probably colonies in which the identity of the individual granules has become lost as a result of intensive staining particularly of the matrix

Much as I dislike criticizing the opinions of others I think it is necessary to challenge the statements made in North and Central Africa on the question of certain initial bodies Donatien has described and illustrated so-called initial bodies of *Rickettsia conjunctivae* in the conjunctival epithelial cells of

cytes It is debatable whether they may be said to be degenerate owing to their extraordinary parasitic propensities I prefer simply to call them bacteria, and leave the choice of humiliating adjectives to later investigators. Also if we acknowledge that they are bacteria there is no point in referring to them any longer as viruses Before the facts are overlooked, we must mention that the organisms are nonmotile, nonsporulating, non acid fast and gram negative and that they stain rather lightly with the usual aniline dyes.

Perhaps I am wrong, but I can see no justification for separating the Chlamydozoaceae from the Rickettsiaceae merely because none of the former have been shown to depend on arthropods for their transmission Would we be right to refuse to put *Trypanosoma equiperdum*, not only in the genus *Trypanosoma* but even in the family Trypanosomidae simply because horses become infected as a result of coitus and not due to the bites of tsetse flies? In any system of classification morphological considerations must surely come first and, so far, I have failed to discern any possible dividing line between the Rickettsiaceae and the Chlamydozoaceae It is easy to put a single colony of organisms stained with Giemsa under the microscope, and defy anybody to identify them as *Coxiella burnetii Cowdria ruminantium, R canis R bovis* or one of the accepted causal agents of salmon "poisoning" ovine enzootic abortion psittacosis trachoma and conjunctivitis of cattle While we are on this question it is as well to remember that *Coxiella burnetii* does not rely too exclusively on arthropod vectors for its transmission If we are going to divide rickettsias on the basis of arthropod and nonarthropod transmission, what are we going to do with the causal organism of salmon "poisoning" which has been so inconsiderate as to select as its natural carrier the small intestinal fluke *Troglotrema salmincola* After weighing the pros and cons I believe that we should abolish the family Chlamydozoaceae In any case the name is misleading, owing to its animal taint

The individual granules or rickettsias vary in size from minute coccoid forms of about 0 2 μ to relatively large oval or ring shaped bodies of approximately 2 μ The degree of pleomorphism is astounding and we also find large and small bipolar forms moderately large and small bacillary and coccal forms and horse shoe boomerang open ring, and triangular forms The granules frequently stain uniformly but some have relatively clear centers Many rickettsias manifest narrow chromophobic halos Probably all genera but one are characterized by the development at some time or other of bipolar and small coccal and bacillary forms So far the exception the large apparently nonpathogenic genus found in different parts of the world scattered diffusely in the conjunctival epithelial cells of sheep cattle, and goats, seems to occur only as large oval initial bodies 2 μ in diameter Further study may convince us that even this largest member of the community of rickettsias occasionally exists as a smaller organism

The larger rickettsial granules are initial bodies the smaller elementary bodies Between are the intermediate forms Generally speaking initial bodies stain blue with Giemsa, but many of the largest granules of *Cowdria ruminantium* for instance take on a reddish hue Elementary bodies usually

Proposed Classification of the Rickettsiaceae

(1) *Rickettsia* This genus should include organisms with antigenic factors common with *Proteus* strains whether they are transmitted by arthropods or not Presumably the genus will include *R prowazekii R typhi R rickettsii R conorii R tsutsugamushi* and probably *R akari*

(2) *Coxiella* The only known species is *C burnetii*

(3) *Cowdria* *C ruminantium* is the only recognized species

(4) *Ehrlichia* Valid species seem to be *E canis E bovis* and *E ovina* Ranham's pigeon organism probably should be included Other possible candidates are the causal agent of tick borne fever of sheep and the rickettsia found by Donatien and Gayot to produce a fatal swine disease in North Africa resembling heartwater of ruminants

It has been suggested that the rickettsia of salmon poisoning may be an *Ehrlichia* I cannot agree

(5) *Chlamydozoon* Because this name implies membership of the animal kingdom I prefer to call the genus *Prowazekia* if this is permissible The genus is reserved for the organisms responsible for trachoma and inclusion blennorrhoea They do not grow in eggs The matrix of the inclusion contains glycogen

(6) *Miyagawanella* Dr Meyer is more capable than I am of dealing with this genus I should however like to make sure of the inclusion of the harmless intestinal rickettsia recently described by Baker in calves as well as the causal agent of ovine enzootic abortion Also it might not be out of place to question the wisdom of separating ornithosis from psittacosis when intermediate types of virus are to be found Dr Edward holds the same view

(7) *Colesiota* *C conjunctivae* is the only definite species

(8) *New Genus* This is necessary to provide a home for the very large harmless rickettsia found in the conjunctival epithelium of sheep goats and cattle There is no cross-immunity between it and *C conjunctivae*

Until more is known about them I consider that *Rickettsia quintana* the supposedly pathogenic rickettsias of the conjunctival epithelial cells of the ox goat pig fowl and possibly the dog and the causal agent of salmon poisoning cannot be assigned with certainty to any particular genera *Rickettsia lestoquardi* is most probably *Rickettsia conjunctivae suis* Whether there is such an organism as *Rickettsia avium* seems to be open to doubt Kurloff's bodies definitely are not rickettsias

In this short space I have endeavored to cover a wide field and I realize only too well that the task has been performed imperfectly There has been no space for the language of diplomacy and I trust that my dogmatism has not been taken too much amiss

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[illegible]

sheep as spherical homogeneous masses 5 to 20 μ in diameter and staining light red with May Grunwald Giemsa I am very familiar with these balloon-like structures that also often repose in depressions on the surface of the nucleus They have nothing to do with any rickettsia but future research may prove that they are colonies of a new member of the pox group

Donatien and Gayot reported similar large, round reddish bodies 5 to 15 μ in size, in association with rickettsial conjunctivitis of pigs and Malbrant followed suit by describing identical inclusions of 2 to 10 μ in diameter in the conjunctival epithelial cells of dogs in Brazzaville I have not seen these large inclusions in pigs and dogs myself Obviously, they constitute an interesting field for study

Having trodden gently on the toes of others, I must indulge in a little self criticism It was not very sensible to name the organisms associated with conjunctivitis in the various domestic animals, *Rickettsia conjunctivae ovis* *R conjunctivae caprae* and so on, because people have been misled into allocating all these to the genus *Colesiota* There is much similarity between the supposedly pathogenic conjunctival rickettsias of the ox, goat, pig and fowl, but that of the sheep is in a category all of its own and reminiscent in some ways of *Miyagawanella psittaci* and in others of *Cowdria ruminantium* Although all the available evidence points to these eye rickettsias of the ox goat, pig and fowl as different species probably of the same genus I feel that further research should be undertaken in the light of recent experience I had virtually no success in infecting healthy eyes of fowls merely by dropping infected lachrymal secretion into them but the disease was produced readily by rubbing a pledget of cotton wool stiffly over the inflamed conjunctiva and then, just as firmly, over the inner surface of the eyelid Likewise it is almost impossible to infect one ox from another Cross infection experiments ought to be carried out involving vigorous rubbing of the diseased and healthy conjunctival membranes before refuting the possibility of two or more of these rickettsias being the same Serological tests also may be feasible

The conjunctival epithelial rickettsias of the domestic animals thus fall into at least three genera First we have the established genus *Colesiota* the only known species of which is *Colesiota conjunctivae* responsible for conjunctivitis of sheep Then we have to find generic and specific names for the very large and apparently nonpathogenic rickettsias found in the conjunctival epithelium of sheep For the time being, it may be convenient to regard the similar organisms occurring in goats and cattle as members of the same species Finally we shall probably have to create one generic and four specific names for the other conjunctival rickettsias affecting the ox pig goat, and fowl but there is no frantic hurry for such action The old descriptive names such as *Rickettsia conjunctivae suis*, will serve our purpose for the time being

Before suggesting a tentative classification of the rickettsias I should like to question the desirability of relying on filterability as a criterion Provided the attendant circumstances are right, a small proportion of the minute coccal and bacillary forms may be expected to pass through Fortunately, no harm in this respect has been occasioned and the genus *Coxiella* will remain even though we do not refer again to the property of filterability

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PLATE 1

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3 Psittacos M pl M ed t f d f m t ry b f 14 IX
4 P ttac M u pl M ly b pola t l bod 1400X
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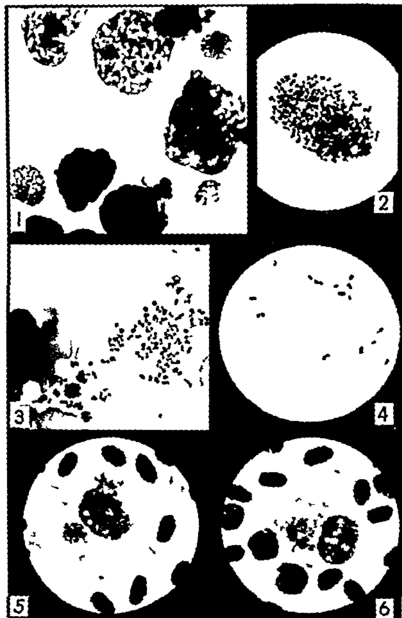
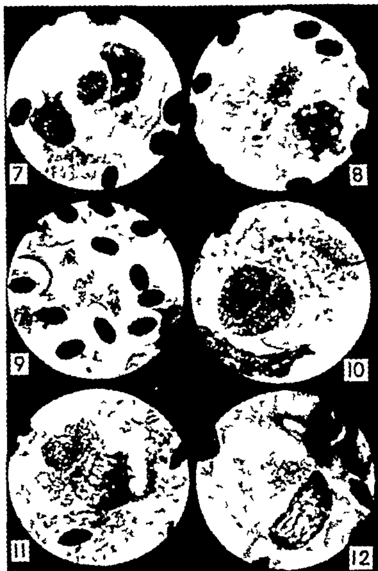


Plate 1



PLA 2

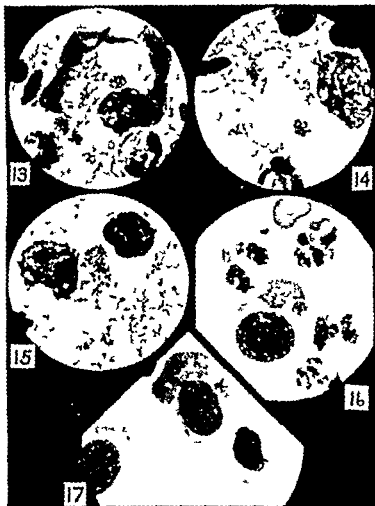
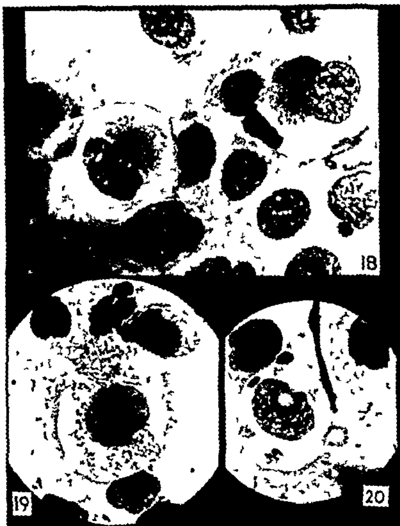


PLATE 3



P 4

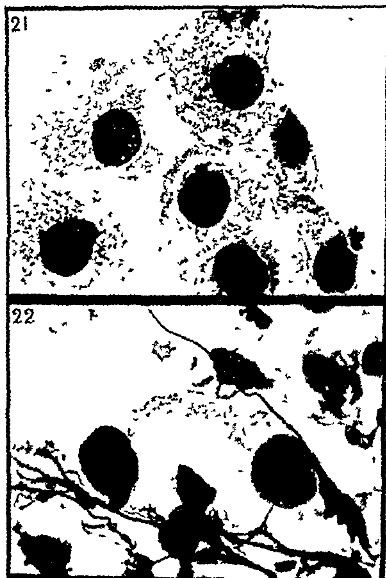
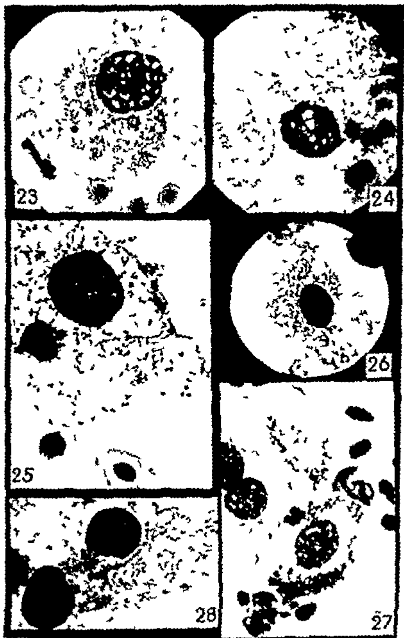
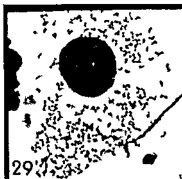


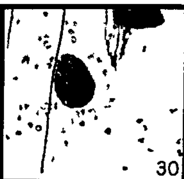
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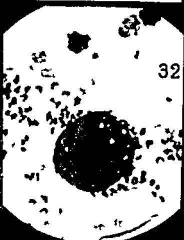
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PLA 7











PLATE 4







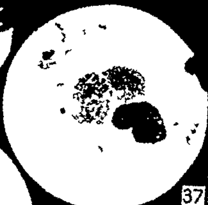
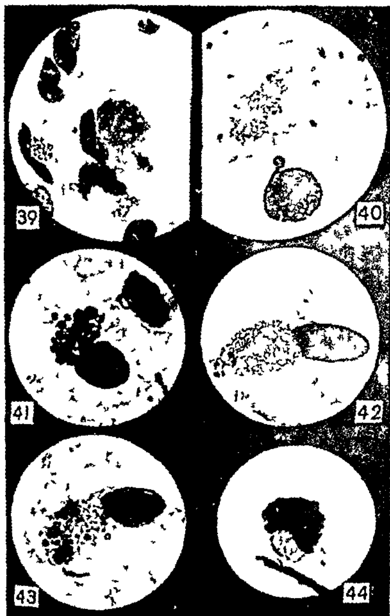




PLATE 6


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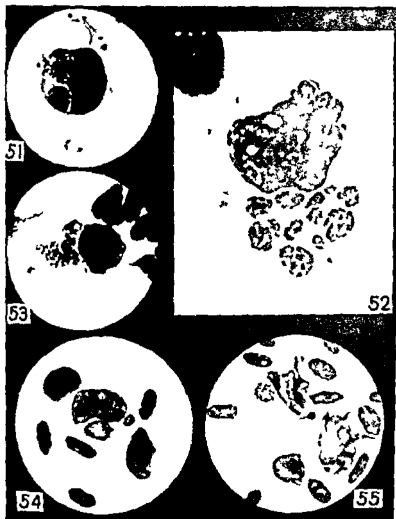


PLATE II

NOMENCLATURE OF THE RICKETTSIACEAE PATHOGENIC TO VERTEBRATES

By Cornelius B Philip

Federal Security Agency Public Health Service National Institutes of Health National Microbiological Institute and Rocky Mountain Laboratory Hamilton Montana

In recent years names for rickettsial agents of animal diseases too frequently have been published without regard to prior proposals or to accepted and reasonable systematic procedures Any author for protection and endurance of his own proposals should pay attention to these essentials of standard practice It is inevitable that not all newly proposed names for rickettsial agents can have equivalent initial characterization and that laboratory clinical and epidemiological information will be in various states of completeness for older names often depending on the professional interests of early investigators It is also inevitable that opinions of specialists on the group will vary concerning systematic levels (specific and supraspecific names) that should be recognized on technical as well as nomenclatorial grounds This can be expected since it is comparable to what has happened in the past systematic organization of other so-called higher biological groups Too often nomenclature has been considered as an end in itself and not as a tool for reference to a given group of biologically similar organisms Especially applicable here is Andrewes s (1951) statement ' Systems of nomenclature are for man s convenience and cannot hope to be wholly logical as to represent faithfully the evolution of all living things * It is pertinent to add that the systematist must *set up arbitrarily a comparatively static system of specific epithets to represent* what are in effect the dynamic and changing results of evolution among members of a given group in other words we unavoidably accept *Rickettsia prowazeki R rickettsiae* and *R tsutsugamushi* as of taxonomic categorical equivalence in spite of biological evidence that they are not precisely on the same evolutionary plane

The increasing facility of laboratory methods and diagnostic aids for differentiating and comparing the biological characteristics of rickettsial agents has been responsible for some synonymy but carelessness in systematic practices has also resulted in too great a proportion of invalidated rickettsial names This leads to confusion discredit to sound nomenclatural practice in general and the understandable impatience among medical and technical personnel which results in perpetuation of colloquial and often cumbersome common names in different languages Nevertheless there is no adequate substitute for the precise bibliographic reference to the rickettsial agents of disease provided by application *of the binomial system and there is no question of its* utility as applied to this group of pathogens

The writer is not proposing a system of classification for this group of agents but is reviewing names already in the literature (notably by Pinkerton 1936

* F th m P ple 8 f th Cod t t Th p m ry p rpos f gi g m t t om g p n t t d t th h t rs f th h t ry f th g p b t t pply m f f rr g t t Th trad t th biect of s m l g t th t u t l phyl g t c l t h p th s g g oup d t t om c y t m f b m l t t lly b ppl d

1947 Philip 1943 Steinhaus 1946 Macchiavello 1947 Anigstein 1947 Bengtson 1948) and their systematic relationships with some personal opinion as to application of priority and categorical levels. Certain difficulties that have delayed general acceptance of a similar classification for the viruses were discussed at the conference on which this monograph is based and elsewhere There are equally evident flaws in the numerous definitions that seek to decide where the boundary between the rickettsias and the viruses should be drawn Andrewes (1951) has most recently reviewed this problem and his goal of keeping our feet on 'the solid ground of practical convenience is laudable The writer is not qualified to discuss this controversial border line field and will confine himself strictly to discussion of agents now classified in Pinkerton s family Rickettsiaceae under the recent concept of Bengtson (1948) However since the goal of systematic stabilization is closer for this group than for the viruses it is his opinion that any recommendations regarding the setting of a future date of validation for binomial names of viruses should either exclude the presently conceived rickettsias or accept the present validly published rickettsial binomials with original dates and authors

The genus *Rickettsia* was first proposed by da Rocha Lima (1916) for the agent of European or epidemic typhus fever with *Rickettsia prowazeki* as the only species Nomenclatorially therefore it is monotypic and other agents assigned to the genus depend on subsequent opinions of the enlarged or restricted characteristics of the genus with relation to the genotype species Pinkerton (1947) Andrewes (1951) and Rake at this Conference in particular have discussed criteria for such estimation Not only were later-discovered typhus like arthropod borne agents included but within a decade apparently similar types of organisms with no known pathogenicity for vertebrates were found to be symbiotically associated with various arthropods and these were also assigned to *Rickettsia* as reviewed among others by Cowdry (1926) and more recently by Steinhaus (1946) The latter author has logically pointed out that contrary to some opinion pathogenicity for vertebrates should not be a restrictive attribute of the genus and Cowdry has stated The balance between symbiosis and parasitism is a delicate one which may be upset by factors largely unknown referring to forms in the human flea the sandfly and the mosquito Since intimate association with arthropod tissues is another attribute in most modern definitions that follows the character of the genotype species there may be more reason for supraspecific separation of agents originally assigned to *Rickettsia* which parasitize conjunctival epithelium or elements of circulating blood of several kinds of vertebrates including birds and fishes often without apparent need for arthropod intervention

Much information is still lacking for satisfactory comparative classification for many of these organisms Rake (1948) has transferred some of them to his genus *Colesiota* and Bengtson (1948) has provisionally treated others in appendices to her system of Rickettsiaceae One of these *R canis* Donatien and Lestoquard was designated as genotype when Moshkovsky (1947) split off his new genus *Ehrlichia* and erected his family Ehrlichiaceae in which family he also placed *Cowdria ruminantium* (Cowdry) Moshkovsky of bovine heartwater

and his *Ehrlichia (Rickettsia) kurlovi* ' (the parasitic nature of which in monocytes of guinea pigs and other animals has been questioned, but which he suggested should have an arthropod vector) It should further be mentioned that he provided a tentative classification of what he termed the "cytotropic viruses of the Chlamydozoa Rickettsia Group Although he states that the heartwater agent ' differs from the classical Rickettsiae and agrees with the organisms of the family Ehrlichiaceae in its dimensions in sensibility toward the action of the sulfonamide drugs and especially in its aptitude to provoke the formation of vacuoles in the host cell, including the aggregated elementary bodies representing the parasite ' he goes on to raise *Cowdria* 'to the rank of a genus intermediate between the families Rickettsiaceae and Ehrlichiaceae " The taxonomic impropriety of his apparent synonymizing the earlier *Miyagawanella* (misspelled *Myiagawanella*) Brumpt of Lymphogranuloma venereum with his subgenus *Ehrlichia* is not of concern here, but the respective genotype species are not accepted as congeneric by Rake (1948)

Though there appears to be justification for accepting *Ehrlichia* for *R. bovis* and its tick borne relatives the incomplete information about these agents would not appear to warrant separation on a family level in spite of parallel action by Rake (1948) in transferring *Rickettsia conjunctivae* to *Colesiota* n gen in Chlamydozoaceae Moshkovsky In consideration of such high level splitting morphological similarities of the intracytoplasmic 'inclusions' morulae ' or spherical colonies of tightly packed rods of even *R prowazekii* itself observed by Mooser (1945a) and others in resistant hosts should be recalled. It would seem more rational, at present, for those desiring suprageneric groupings here to place *Rickettsia* and *Coxiella* in a Tribe Rickettsiaceae and *Ehrlichia* and *Cowdria* in a Tribe Ehrlichieae

If Moshkovsky s family Chlamydozoaceae were to be abandoned as has his Ehrlichaceae and the elements now in *Chlamydozoon* and *Colesiota* returned to the Rickettsiaceae under the tribal conception they would be assigned logically either to the above latter Tribe or perhaps another erected in which no arthropod relationship is accounted for This however would eliminate one of the few remaining restrictive characters of the family namely, the arthropod vectorship

Such a course is not followed in the system below pending more complete knowledge to justify it The entire group had been placed in the Class Schizomycetes (Steinhaus 1946) although this category was left unassigned in Bergey s Manual (1948) because of the still uncertain taxonomic affinities of these organisms

Order RICKETTSIALES Buchanan and Buchanan 1938

Family RICKETTSIACEAE Pinkerton 1936

Genus I *Rickettsia* da Rocha Lima 1916 (type genus of family) Syn *Strickeria* Stempell 1916* *Fossilis* Megaw (1943) (provisionally published and therefore not valid under Rule 12)

* Stempell gen d spec es ted t *Rickettsia prowazeki* d Rocha Lim by ov a month It stra g th t thi pri m h be n overlooked o l ng d to p ev t confus request has be bm tted t th Int rn t l Comm t ppre s t (Ph l p 1952)

Subgenus A *Rickettsia* Philip 1943 A typical subgenus is required if other subgeneric names are to be used (Rule 5 of the Code)

Species 1 *prowazekii* da Rocha Lima (genotype species by monotypy) Syn *Strickeria jurgensi* Stempell 1916* *R kairo* da Rocha Lima 1930 *R exanthematotyphi* Kodama 1932 *R prowazeki* var *prowazeki* Pinkerton 1936 *R. prowazekii* subsp *prowazekii* Philip 1943 *R. altiplanica* Veintemillas 1944 [Louse borne epidemic or European typhus and recrudescent in Brill s disease]

Species 2 *typhi* (Wolbach and Todd 1920) Philip 1943 Syn *F manchuriae* Kodama Takahashi and Kono 1931 (not *mandchuriae* Macchiavello 1947) *R mooseri* Monteiro 1931 *R exanthematofebri* Kodama 1932, *R muricola* Monteiro and Fonseca 1932 R "*murina* or *fletcheri* Megaw 1935 (? invalid Rule 12) *R prowazeki* var *mooseri* Pinkerton 1936 *R prowazekii* subsp *typhi* Philip 1943 *R murina mooseri* Veintemillas 1944 [Flea borne murine or endemic typhus]

Mooser (1948) has taken vigorous exception to the possibility that Wolbach and Todd could have distinguished pathologically epidemic from murine typhus in proposing the name *Dermacentroxenus typhi* in 1920 This is true but as he himself admits (1948b p 74) over a decade passed with much refinement in methodology before it was realized that Mexican typhus was in reality a mixture of both When Wolbach and Todd thought they were describing and figuring a different agent than Old World typhus there is no proof that they actually did not study some cases of endemic (Mexican as they thought) typhus which is practically the same reasoning by which Hayashi s *Theileria tsutsugamushi* in the same year is now accepted also by generic transfer as the prior specific name for the agent of tsutsugamushi disease There would be much more doubt of the alternative of synonymizing *D typhi* with *R prowazekii* (of which the authors were cognizant) and even the latter was only adequately characterized after much refinement of techniques It may even be flea borne as Mooser has postulated in several articles while the former may become epidemic like by secondary louse passage There is some experimental support for this

Subgenus B *Zinsserra* Macchiavello 1947 (reduced from genus herewith) The writer is scarcely convinced that the characters of trombiculid mite transmission and OXK serology are more than specific but Steinhaus (1946) considers it to have characteristics sufficiently different from the type species *R prowazekii* to warrant giving it a generic differentiation and Macchiavello so treated it Topping and Shepard (1947) consider the unique difference from all other rickettsias of separation in the interzone during ether treatment as of specific value but this probably strengthens the supraspecific differentiation

Species 3 *tsutsugamushi* (Hayashi 1920 genus *Theileria*) Ogata 1931 He first used combination of *Rickettsia tsutsugamushi* but proposed it independently for strains he was studying not as a

reviser of Hayashi's name (Subgenotype species by monotypy) Syn *R orientalis* Nagayo Tamiya Mitamura and Sato 1930 *R akamushi* Kawamura and Imagawa *1931* *R orientalis* var *schüffneri* Amaral and Monteiro 1932 *R megawi* Amaral and Monteiro 1932, *R megawi* var *fletcheri* Amaral and Monteiro 1932 *R tsutsugamushi orientalis* Kawamura 1934 *R pseudotyphi* Versvoort 1938, *R sumatranus* Kouwenaar and Wolff 1939, *Dermacentroxenus orientalis* Moshkovsky 1945, *R orientalis* var *tropica* Hayakawa and Hokari 1947 [trombiculid mite borne tsutsugamushi disease or scrub typhus]

R nipponica Sellards (1923) has generally been discredited because of artificial cultivation since the strain has been lost and it was the only species (therefore monotype) mentioned by da Rocha Lima (1930) in erecting his genus *Rickettsioides* it will be difficult to assign any further species as congeneric with *R nipponica* If this should ever be reinstated in synonymy of *R tsutsugamushi* then *Rickettsioides* would take precedence over *Zinssera*

Subgenus C *Dermacentroxenus* Wolbach 1919 (genus reduced to subgenus by Philip 1943) Treated as full genus by Pinkerton (1936), Steinhaus (1946) and others but completely synonymized by Brumpt (1922) and Bengtson (1948) Its subgeneric acceptance appears warranted for the growing group of rickettsial agents capable of parasitizing the nuclei of certain host cells Topping and Shepard (1947) have reviewed laboratory evidence for this as a subgroup All but one species are tick borne

Species 4 *rickettsii* (Wolbach 1919 *Dermacentroxenus*) Brumpt 1922 (subgenotype species by monotypy in original genus) Syn *R brasiliensis* Monteiro 1931 *R typhi* Amaral and Monteiro 1933 (not Wolbach and Todd 1920) *Dermacentroxenus rickettsi* var *brasiliensis* Pinkerton 1936 [Tick borne agent of American spotted fever]

Since Amaral and Monteiro were mistaken in assigning *R typhi* to 'eastern type spotted fever this does not constitute correct prior revision of generic status

Species 5 *conorii* Brumpt 1932 Syn *R blanci* Caminopetros 1933 *R megawi* var *pijperi* Mason and Alexander 1939 *D conori* Steinhaus 1946 [Tick borne agent of fièvre boutonneuse South African tick bite fever and probably Kenya typhus and Indian tick typhus]

Species 6 *australis* Philip 1950 [Agent of North Queensland tick typhus tick transmission is presumed but not proved]

Species 7 *akari* Huebner Jellison and Pomerantz 1946 [dermanyssine mite borne rickettsialpox] Philip and Hughes (1948) pointed out the relationship of this subgenerically while several recent cases have been reported with OX_{19} serology (Rose correspondence)

Subgenus *incertae sedis*

Species 8 *quintana* Schminke 1917 Syn *R wolhynica* Jungmann

Genus III *Cowdria* (Moshkovsky) Moshkovsky 1947 (Rule 16 antedates Bengtson 1948 first proposed by Moshk as subgenus 1945) Syn. *Nicollea* Macchiavello 1947 (genotype species identical) Even if prior date of actual publication of the latter could be shown which is doubtful it could not supersede the subgeneric name for the same type species the author also placed *R canis* D & L under this genus Macchiavello further proposed a genus *Cowdryia* for a group of insect symbionts It is believed even if valid, this name would fall by homonomy since this slight orthographic variant would be confusing and hardly conserved under Rules 25 (4) 'note" and 27 of the Bacteriological Code Its questionable differentiation from *Wolbachia* Hertig and many irregularities in included specific epithets are not within the scope of this paper

- Species 1 *ruminantium* (Cowdry 1925) Moshkovsky 1947 (genotype species by monotypy) Syn *R. ruminantium* Cowdry 1925 [Tick borne heartwater of ruminants]

Genus IV *Coxiella* (Philip) Philip 1948 (Rule 16 first proposed as subgenus 1943 antedates Bengtson 1948 but corrected in second printing) Syn *Burnetia* and subgenus *Dyera* Macchiavello 1947 (genotype species of all identical furthermore *Dyera* is invalid since only a typical subgenus *Burnetia* could contain the genotype species under Rules 5 and 20)

- Species 1 *burnetii* (Derrick 1939) Philip 1948 (genotype species by original designation) Syn *R burneti* Derrick 1939, *R. diaporica* Cox 1939, *R (Coxiella) burneti* Philip 1943, *R burneti* var *americana* Anon 1942, *R burneti* var *caprina* Caminopetros 1949 *R burneti* var *henzerling* Kausche and Sheris 1951 [Contaminative and sometimes tick borne agent of Q fever]

The observation of Topping and Shepard (1947) that this is distinctive in not providing a soluble antigen when processed with ether would appear to be of generic significance However Ormsbee (to be published) has produced small amounts of a soluble antigen from *C burnetii* during ether treatment

Discussion

It has not been possible in the time and space allotted to discuss more than incidentally the biological reasons for the above systematic arrangement but in the main they have already been provided by Pinkerton Steinhaus, Bengtson, and others It is believed a compact group of related agents is retained in *Rickettsia* while separating more distantly related species into three other genera Mooser (1945b) has discussed the reasons why customary vector reference to louse borne epidemic and flea borne endemic typhus are only relative terms but he further emphasized the rickettsial species differences This was further reiterated by Topping and Shepard (1947) who review new laboratory evidence bearing on classification of pathogenic rickettsias

In view of the tendency toward refinement of supraspecific concepts as new information accumulates it is pertinent to reiterate that an author should

inquire in proposing new genera if bibliographic reference is facilitated rather than complicated by superficial raising of specific criteria to generic levels so that a definition of the genus *Rickettsia* might ultimately become only a characterization of two close species—*R prowazekii* and *R typhi* While there are plenty of examples of bacteriological genera containing only a single species including two in this paper there is a definite decrease in reference utility as the point is approached where every species of a family becomes a separate genus through continued revision The value of subgenera becomes more apparent under such tendencies Certainly researchers contemplating proposal of any new names would do well to consult either the Bacteriological Code (Buchanan St John Brooks and Breed 1948) or someone familiar with it to insure validity of such proposals

The question of arthropod association would seem a more fundamental consideration in the taxonomic composition of this family than pathogenicity for vertebrates While Andrewes (1951) has stated the class of effective vector is probably not fundamentally important for animal viruses Bengtson (1948) has followed Megaw (1942) in grouping or keying the rickettsiae according to the vectors The spectrum of nonpathogenic symbionts in arthropods is much broader than for pathogenic forms in parasitic species and there are several nonpathogenic species described even in vectors of rickettsial pathogens Since the time of Nicolle it has seemed logical that the genotype species had its evolutionary origin in a parasitic arthropod The more primitive adaptation would appear to be in acarine hosts There may well be question therefore of the congeneracy of agents with plainly no such association at least at some stage in their propagation This does not mean that such an obviously rickettsial agent as that of North Queensland tick typhus should be excluded until the arthropod vector relationship is proven nor yet Q fever which is also adapted to extra arthropod dissemination but it does give a more rational basis for high level separation of such forms as *Colesiota conjunctivae* (Coles) Rake and *Miyagawanella* spp

The qualitative differences on a specific level such as the lack of antigenic homogeneity of strains of *R tsutsugamushi* reviewed by Topping and Shepard (1946) compared to the homogeneity of isolates of *R prowazekii* and of *R typhi* are to be expected just as in other systematized biological groups which we consider on the same categorical although not evolutionary level Such considerations strengthen rather than weaken the utility of the binomial system for general reference of the rickettsial agents even though it is entirely possible a reviewer some decades hence might suggest that the Karp and Gilliam strains of the first actually represent taxonomic subspecies, since serologically they appear about as far apart as *R prowazekii* and *R. typhi* It would not be easy for such a reviewer to explain how his two subspecies could infect different members of the same Japanese family contracting the disease on the same farm in successive years (Philip 1947 TABLE 3) The utility of subspecies based on lack of antigenic homogeneity or on differences of sensitivity of antigens as in Q fever is not apparent at present

It is hoped that the review of rickettsial classification offered above is neither too radical nor too conservative to furnish the utility desired for adequate taxonomic reference to pathogens in vertebrates that are at least presumed to be arthropod borne while showing some idea of natural relationships as far as present information permits

Summary

The family Rickettsiaceae pathogenic for vertebrates and arthropod adapted on the basis of present technical and nomenclatorial information is considered to comprise four genera—*Rickettsia Ehrlichia Cowdria* and *Coxiella* Seven species fall into three subgenera of the first namely *Rickettsia* (*Rickettsia*) *prowazekii* (genotype species) *R* (*R*) *typhi* *R* (*Zinssera*) *tsutsugamushi* *R* (*Dermacentroxenus*) *rickettsii* *R* (*D*) *conorii* *R* (*D*) *australis* and *R* (*D*) *akari* plus three *incertae sedis*—*R* *quintana* *R* (?) *melophagi* and *R* *suis* *Ehrlichia* contains *E* *canis* (genotype species) *E* *bovis* *E* (?) *ovina* and *E* (?) *kurlovi* The other two genera are monotypic for *Cowdria ruminantium* and *Coxiella burnetii* respectively Many names are included in synonymy partly it is pointed out because of inattention to the Bacteriological Rules of Nomenclature

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ON THE NOMENCLATURE AND CLASSIFICATION OF INSECT VIRUSES*

By G. H. Bergold
Laboratory of Insect Pathology, Sault Ste. Marie, Ontario, Canada

Virus diseases of insects have been known and studied intensively since about the middle of the nineteenth century and such famous bacteriologists as Pasteur and von Prowazek were among their early investigators. The recent development of biophysical methods encouraged a new search for the infectious agent. Yet before having knowledge of isolation of any insect virus Holmes (1948) adopted some names from the literature, invented several new ones, and classified them into two genera with five and one species respectively. This was based on uncritical acceptance of inadequate descriptions of symptoms of diseased insects. Steinhaus (1949) realized the undesirability of this situation and, since in the meantime several viruses had been isolated and described (Bergold 1947, 1948, 1948a), he partially corrected the situation. In a further improvement, Steinhaus (1949a) accepted two genera, *Borrelina* (with six species) and *Morator* (with one species), from Holmes and added two new ones, *Paillotella* (with one species) and *Bergoldia* (with five species).

What is the general feeling about naming and classifying insect viruses? At present there is absolutely no necessity for doing so. Yet a start has been made and we now have intensive knowledge of several insect viruses. The relatively highly differentiated morphology of insect viruses, with their complicated development and multiplication cycles, indicate that they are organisms (Bergold 1950). The use of binomials therefore is justified.

For the description of genera and species seven of the eight criteria proposed by the virus subcommittee at the Fifth International Congress for Microbiology in Rio de Janeiro in 1950 (Andrewes 1951) can be well applied. *Criterion 1*—Morphology and methods of reproduction are quite characteristic for insect viruses (Bergold 1950, 1952a, 1953). *Criterion 2*—Chemical composition and physical properties may be important. Investigations of base ratios of the nucleic acid of several insect viruses (Wyatt 1952) indicate differences between viruses from different hosts. *Criteria 3-5*—Immunological properties, susceptibility to physical and chemical agents, and natural methods of transmission have not been investigated intensively. *Criterion 6*—Host, tissue and cell tropisms are very important characters of insect viruses, which are probably the most host-specific of all known viruses. *Criterion 7*—Pathology (including inclusion body formation) is also of great significance since size and shape of the inclusion bodies are valuable characters for distinguishing the genera. *Criterion 8*—Symptomatology is practically of no value.

For all these reasons naming and classifying insect viruses (not diseases!) may be encouraged, under the following conditions:

(1) An insect virus must be isolated and demonstrated by the electron microscope before any name or classification is applied.

* Contribution No. 41, Division of Forest Biology, Science Service, Dept. of Agriculture, Ottawa, Canada.

(2) No "tentative" or "provisional" names or groups should be established until the virus itself has been demonstrated, no matter how obvious any symptoms of disease may be. Such tentative proposals only confuse the student and annoy the expert.

(3) Description of a genus should be concise, and be based mainly on the virus itself.

If these three conditions are accepted, the genera *Paillotella* and *Morator* have to be dropped. The genus *Paillotella* is based on light microscopic observations in 1926 by Paillot (1926) of small granules less than 0.1 μ in diameter in diseased larvae and pupae of *Pieris brassicae* (L.). Polymorphic inclusion bodies were also present. Steinhaus (1949a) is not certain whether Paillot saw the actual virus and justified his acceptance of a virus species, so inadequately described, in the following words: "In order to preserve continuity, however, it is suggested that, at least for the time being, all those species of insect viruses, which have been previously recognized as such by Holmes in Bergey's *Manual of Determinative Bacteriology* be accepted and retained, even though some of them may not as yet have been seen with the electron microscope or demonstrated by a determination of their physical properties." In the case of *Morator*, a possible virus disease of honey bee larvae, the situation is even worse (White, 1913, 1917). Nothing has been seen, even with the light microscope, neither inclusion bodies nor virus particles. There is not even evidence of infectivity.

For these reasons and furthermore as *nomina dubia* (Art. 63 of the code) the generic names *Paillotella* and *Morator* must be rejected.

This leaves the genera *Borrelina* and *Bergoldia*. The generic name *Borrelina* was originally suggested by Paillot (1926) for the causative agent of three different diseases: (1) *Borrelina bombycis* (= *Borrelina bombycis* Paillot), causing the polyhedral disease of *Bombyx mori*; (2) *Borrelina pieris* (= *Paillotella pieris* (Paillot) Steinhaus), causing a disease of *Pieris brassicae* (L.) characterized by polymorphic inclusions in the cytoplasm of host cells (virus not demonstrated); (3) *Borrelina brassicae* (= *Bergoldia brassicae* (Paillot) Steinhaus), causing the granulosis disease of *Pieris brassicae* (L.) (virus not demonstrated). The fact that the original description of the genus *Borrelina* given by Paillot (1926) was such that it could be applied to the agents of three different diseases invalidates the description for any one of them. Furthermore, the description of *Borrelina bombycis* is not adequate. As the causative agent Paillot (1926) described minute granules visible under the dark field microscope in blood and tissue of diseased insects. Glaser and Cowdry (1928), however, found that granules similar to those seen in blood of diseased larvae are also present in the blood of healthy ones. Steinhaus (1949) accepted the name *Borrelina bombycis* from Holmes on the assumption that Paillot had seen the virus. Yet intensive investigations carried out by the author indicate that it is very difficult to identify virus particles in diseased silkworm serum even with the electron microscope.

The following names have been proposed for the causative agent of the silkworm jaundice: *Micrococcus bombycis* (Béchamp) Cohn, 1872; *Microsporidium polyedricum* Bolle, 1894; *Micrococcus lardarius* Krassilstschik, 1896; *Chlamy*

[illegible]zoon bombycis von Prowazek 190[illegible] *Crystall plasma p[illegible]lyedricum* P[illegible] [illegible]rell 1918 *Borrelina bombycis* Paillot 1926 Since all the de[illegible] on which the above names are based are inadequate there was no justification [illegible] Holmes to choose *Borrelina* violating the rule of priority Therefore in [illegible] to overcome the general confusion the name *Borrelina* is rejected a[illegible] entirely new name now proposed

Almost all the knowledge we have at present of insect vir[illegible] *rus particles liberated from polyhedral bodies or capsules* [illegible] particles seen in the serum The first to assume that polyhedr[illegible] the carriers of the infectious agent was Bolle (1894) He [illegible] hedral body a a multiplication form and resting stage [illegible] named *Microsporidium polyedricum* Bolle (1898) investi[illegible] polyhedral bodies and was the first to find that they dissolv[illegible] alkaline gut juice of the larvae as well as in several alkali and [illegible] He observed that the polyhedra consist of a central granulated [illegible] tougher peripheral layer surrounded by a thin membran[illegible] Bolle [illegible] the polyhedral bodies are protein in nature and conta[illegible] lipoid substan[illegible]

The author considers that these fundamental results [illegible] Bolle [illegible] years ago which provided the basis for the final isolation and identi[illegible] of insect viruses (Bergold 1947) deserve the namin[illegible] of the [illegible] The name *Microsporidium polyedricum* Bolle ha[illegible] to be rej[illegible] *ambiguum* (Art 62 of the code) The author therefore pr[illegible] the name *Bollea nomen novum* = *Borrelina* Paillot

It should be mentioned in this connection that although the [illegible] code does apply to the virus field no priority date for virus names has been set (Conference on Virus and Rickettsial Classification and Nomenclature New York January 1952*) Therefore no published virus names are actually valid at present.

In addition to the genera *Bollea nom nov* and *Bergoldia* Steinhaus the author would like to propose a new genus *Smithia gen nov* for the spherical viruses recently isolated and demonstrated by K M Smith and R W G Wyckoff (Smith and Wyckoff 1950 1951)

The descriptions of the genera *Borrelina* Paillot (= *Bollea* Bergold) and *Bergoldia* Steinhaus given by Steinhaus (1949 1949a) can be improved in view of our present knowledge

For separation of the genera criteria one [illegible] and seven of the Rio plan are sufficient but differences on the other criteria may be present and could be included later In the description of a species there is little hope that any insect virus may be defined by any single criterion There will often be overlapping of some properties in some species We do not know at present whether experience in and the rules of the general field of microbiology can be applied to the concept of the virus species

The method of describing the virus species given by Steinhaus (1949 1949a) and by the author (below) is quite unorthodox and very long It contains details which seem to be of dubious value at present but may be of importance

*This is th[illegible] Conference on wh[illegible] [illegible] chart b[illegible]

in the future Nevertheless the author proposes that, pending further knowledge which will enable one to set a precise borderline for the separation of virus species the eight criteria of the Rio plan be adopted as a basis for a standard description In that way deletion and addition based on advances in knowledge can be easily applied and the validity of the Rio plan can be checked. The general application of a standard method may also help to coordinate, and standardize research in different laboratories This would provide an easier means for comparison and a better understanding of viruses than isolated research groups could otherwise achieve

A preliminary attempt is made in TABLE 1 *to compare the important charac*ters of insect viruses by applying the Rio plan From this attempt it can be concluded that insect viruses so far examined do not differ greatly in respect to criteria (5) and (8) These two criteria might, therefore be omitted in future descriptions This is also true for criterion (4) if the inclusion bodies containing the virus particles are compared although further research may indicate differences if the free virus particles are treated with different reagents. *It is to be expected that serological investigations will reveal additional* valuable differences between virus species As a whole TABLE 1 indicates that insect virus species can be defined only if several characters are considered and that single ones may be of little or no value

It should be noted that the author purposely rejects the family Borrelinaceae Holmes and the 'sub-order Zoophagineae Holmes (Holmes 1948) It was generally agreed at the ' Conference on Virus and Rickettsial Classification and Nomenclature January, 1952* in New York that, for the time being no attempts to classify viruses in general should be made above the genus level since we have no knowledge of their phylogenetic relationship

Comparing the characters of the species in the genus *Bollea* [for example their differences in multiplication (number of rods in a bundle)] one might consider that the differences are above the species level That would mean that sub-genera genera or new sub-families should be established Yet such differentiation should be postponed until more knowledge has been accumulated

The author proposes the following descriptions for the genera and species of insect viruses

Descriptions of Genera and List of Species

Genus **Bollea** *nom no* = *Borrelina* Paillot 1926

Viruses mostly rod shaped with dimensions of about 20–70 × 200–400 mμ † occurring usually in numbers within dense polyhedral shaped inclusion bodies (polyhedral bodies) of about 0 5–15 μ in diameter Virus particles often carry the developmental membrane which gives the appearance of thick straight rods Developmental membrane easily shed Viruses multiply and develop and polyhedral bodies form in the cell nuclei of insect larvae and occasionally of insect pupae

Type Species *Bollea bombycis* (Paillot) *comb nov* = *Borrelina bombycis* Paillot 1926

* Th th C f re o wh ch th mon graph s b s d
† R g is arb trary d w d th th t f k ow spec es llow g f r pos bl new pec w th diff re t dimens s

Species *Bollea efficien* (Holmes) *comb nov* = *Borrelina efficiens* Holmes 1948

Bollea reprimens (Holmes) *comb nov* = *Borrelina reprimens* Holmes, 1948

Bollea olethria (Steinhaus) *comb nov* = *Borrelina olethria* Steinhaus 1949a

Bollea campeoles (Steinhaus) *comb nov* = *Borrelina campeoles* Steinhaus 1949a

Bollea peremptor (Steinhaus) *comb nov* = *Borrelina peremptor* Steinhaus, 1949a

Bollea fumiferana spec nov

Genus **Bergoldia** Steinhaus, 1949

Viruses mostly rod shaped with dimensions of about 30–70 × 200–400 mμ* without and 50–100 × 200–500 mμ with the developmental membrane Occurring usually singly in dense ellipsoidal shaped inclusion bodies (capsules, granules) of less than 1 μ length Virus particles normally carry the developmental membrane giving the appearance of thick usually slightly curved sausages Developmental membrane not easily shed Viruses multiply and develop and inclusion bodies form in the cytoplasm and/or cell nuclei of insect larvae and occasionally of insect pupae

Type species *Bergoldia calypta* Steinhaus 1949

Species † *Bergoldia daboia* Steinhaus 1949a

Bergoldia lathetica Steinhaus, 1949a

Bergoldia thompsonia Steinhaus 1949a

Bergoldia clistorhabdion Wasser and Steinhaus 1951

Bergoldia nosodes Hughes and Thompson, 1951

Genus **Smithia** *gen nov*

Viruses mostly spherical with a diameter of about 65 mμ ‡ Occurring in numbers in dense polyhedral-shaped inclusion bodies (polyhedral bodies) of about 0 5–15 μ diameter Viruses multiply and develop and polyhedral bodies form in the cell nuclei of insect larvae

Type species *Smithia rotunda spec nov*

Descriptions of the Species

Genus **Bollea** *nom nov* = *Borrelina* Paillot 1926

Bollea bombycis (Paillot) *comb nov* = *Borrelina bombycis* Paillot 1926 Type Species

(1) *Morphology and Methods of Reproduction* Virus rod-shaped averaging about 2 9 mμ long and 40 mμ in diameter Rods surrounded by a thin (intimate) membrane and consist of several spherical sub-units A slender protrusion (about 60 × 10 mμ) extends from one end of the rod Virus develops from small spheres (about 20–40 mμ in diameter) within a developmental mem

* Range is arbitrary and wider than that of known species allowing for possible new species with different dimensions

† Bergoldia [illegible] (P[illegible]) Steinhaus 1949 is rejected see the [illegible] not determined [illegible]

‡ Measurement taken from electron micrograph prepared by the author from polyhedral bodies of [illegible] kindly supplied by [illegible] the diameter of only one species [illegible] range of diameters [illegible]

TABLE I
COMPARISON OF CHARACTERS OF INSECT VIRUSES
(Criteria of the Rio Plan)

Genus and species	Morphology	Length by diameter mμ	No. of rods/bundle	Chemical composition bases ratio	Immunological properties	Susceptibility to chemical and physical agents	Natural methods of transmission	Tropism		
								Host	Tissue	Cell
Bollea bombycis	Rods	279 x 40	1 to 2	1.34	—	Very stable	Food eggs	*Bombyx mori*	Pantrop	Nucleus
Bollea efficiens	Rods	350 x 5[illegible]	1 (2 or 4)	0.94	—	Very stable	Food eggs	*Lymantria monacha*	Pantrop	Nucleus
Bollea reprimens	Rods	364 x 41	2 (2 or 1)	0.71	—	Very stable	Food eggs	*Porthetria dispar*	Pantrop	Nucleus
Bollea olethria	Rods	290 x 50	Several	—	—	Very stable	Food	*Prodenia praefica*	Pantrop	Nucleus
Bollea campestres	Rods	300 x 40	Several	1.35	—	Very stable	Food	*Colias philodice e[illegible]*	Pantrop	Nucleus
Bollea peremptor	Rods	2[illegible]0 x 30	Several	—	—	Very stable	Food	*Phryganidia californica*	Pantrop	Nucleus
Bollea fumiferana	Rods	260 x 28	1 (2)	0.95	—	Very stable	Food eggs	*Choristoneura fumiferana*	Pantrop	Nucleus
Bergoldia calypta	Rods	257 x 41	1 (2)	1.67	—	Very stable	Food eggs	*Cacoecia murinana*	Pantrop	Cytoplasm
Bergoldia dabois	Rods	340 x 40	1	—	—	Very stable	Food eggs	*Peridroma margaritosa*	Pantrop	Cytoplasm
Bergoldia latt[illegible]etica	Rods	300 x 40	1	—	—	Very stable	Food	*Junonia coenia*	Pantrop	Cytoplasm nucleus?
Bergoldia thompsonia	Rods	2[illegible]0 x 40	1	—	—	Very stable	Food	*Estigmene acrea*	Pantrop	Cytoplasm nucleus
Bergoldia [illegible]storhabd[illegible]	Rods	2[illegible]0 x 50	1	—	—	Very stable	Food	*[illegible]*	Pantrop	—
Bergoldia [illegible]sode[illegible]	Rods	275 x 65	1	—	—	Very stable	Food	*[illegible]*	Pantrop	—
Smithia [illegible]lunda	Spheres	65	o rod	—	—	Very stable	Food eggs	*Saturnia [illegible]*	Pant[illegible]	Nucleus

[illegible]	Ll[illegible] ral mg h	Ll[illegible] or ly: P l h d	C p les	I fec d h l y	h[illegible]: P ly hed	C l les	I mens on: P l hed	C s l	N of pa l: P ly hed	C p les	Alk l M mg ml	M lec l w h of: P l hed	C s l	mp m l ogy
B ll a b b[illegible]	4 × 10⁻	5 × 10⁻	—	6–8	D l h lra	—	5	—	Ma y	—	0 006	378 005	—	0
B lle ffic ens	—	10	—	13 15	Irr f tetra he lra	—	2 5 10	—	Ma y	—	0 006	336 000	—	0
B lle repr m	10⁻	10⁻	—	10–12	Irr	—	1 10	—	M ny	—	0 008	276 000	—	0
B l a l ll	—	—	—	—	Ir	—	2 5	—	Man	—	—	—	—	0
B llea campeol	—	—	—	7	I r	—	1 3	—	Many	—	0 006	—	—	0
B llea perempt n	—	—	—	—	I r	—	1 3 5	—	Many	—	—	—	—	0
B ller fi mifera a	1 ~3 6 × 10⁻	—	—	10	Irr f tetra hed a	—	1 2 5	—	Many	—	0 03	—	300 000	0
Berg ldi e lypta	—	—	—	—	—	Ell [illegible] so dal	—	0 36 x 0 23	—	1	0 03	—	—	0
B g ldi dab ra[b]	—	—	—	—	—	Ell [illegible] so d l	—	0 45 x 0 25	—	1	0 05	—	—	0
Berg ld a lall etica	—	—	—	6–12	—	Ell [illegible] so dal	—	0 45 x 0 30	—	1	0 05[illegible]	—	—	0
Berg ldia ll mps [illegible]	—	—	—	—	—	Ellipso dal	—	0 40 x 0 25	—	1	Lo	—	—	0
Berg ldi cl t l hl n	—	—	—	—	—	Ell [illegible] o lal	—	0 315 x 0 16	—	1	0 04	—	—	0
B g ldia nos fes	—	—	—	10–20	—	Ell [illegible] so dal	—	0 345 x 0 17	—	1	0 01	—	—	0
Smil [illegible] r la	—	—	—	—	Irr	—	0 5–4	—	Many	—	—	—	—	0

[illegible]

TABLE I
COMPARISON OF CHARACTERS OF INSECT VIRUSES
(Criteria of the Rio Plan)

Genus and species	Morphology	Length by diameter mμ	No. of rods/bundle[d]	Chemical composition bases rat	Immunologic l properties[e]	Susceptibility to chemical and physical agents[f]	Natural methods of transmission	Tropism: Host	Tropism: Tissue[g]	Tropism: Cell
Bollea bombycis	Rods	2?9 x 40	1 o 2	1 34	—	Very stable	Food eggs	*Bombyx mori*	Pantrop	Nucleus
Bollea efficiens	Rods	350 x 57	1 f2 o4	0 94	—	Very stable	Food eggs	*Lymantria monacha*	Pantrop	Nucleus
Bollea repriments	Rods	364 x 41	2 f2 f1	0 71	—	Very stable	Food eggs	*Porthetria dispar*	Pantrop	Nucleus
Bollea olethria[b]	Rods	290 x 50	Several	—	—	Very stable	Food	*Prodenia praefica*	Pantrop	Nucleus
Bollea campeoles[b]	Rods	300 x 40	Several	1 35	—	Very stable	Food	*Colias philodice e*	Pantrop	Nucleus
Bollea peremptor[b]	Rods	2?0 x 30	Several	—	—	Very stable	Food	*Phryganidia californica*	Pantrop	Nucleus
Bollea fumiferana	Rods	260 x 28	1 f2	0 95	—	Very stable	Food eggs	*Choristoneura fumiferana*	Pantrop	Nucleus
Bergoldia calypta	Rods	257 x 41	1 12	1 67	—	Very stable	Food eggs	*Cacoecia murinana*	Pantrop	Cytoplasm
Bergoldia dabossi[b]	Rods	340 x 40	1	—	—	Very stable	Food eggs	*Peridroma marg i-tosa*	Pantrop	Cytoplasm nucleus?
Bergold a la he ica[b]	Rods	300 x 40	1	—	—	Very stable	Food	*Ju on a coen a*	Pantrop	Cytopla m nu leus
Bergoldia thompsonia[b]	Rods	2 0 x 40	1	—	—	Very stabl	Food	*Est gmena acr a*	Pantrop	—
Bergoldia cli tori bdion	Rods	250 x 50	1	—	—	Very stabl	Food	*A gyr taen a velu-n na*	Pantrop	—
Berg ld a n od	Rod	275 x 65	1	—	—	Very table	Food	*S bulod ber ta*	Pantrop	Nucleus
Sm thia tund	Sphere	(5	o od	—	—	Very ble	Food x	*A a illica*	Pan r	Nu l

G d ec es	LF ra m b	LD I h	LD C le	I d h	P hed	C es	P hed	C p	N P h d	N C p	A k M s m m	P hec	C es	ym y
B l l b	4 × 10⁻	5 × 10⁻	—	6–8	D lec h d a	—	5	—	M	—	0 006	378 005	—	0
B ll a ff ens	—	10⁻	—	13 15	I f e a he l a	—	2 5–10	—	M y	—	0 006	336 000	—	0
B l a rep m s	10⁻	10⁻	—	10–12	I r	—	1 10	—	Many	—	0 008	276 000	—	0
Bolea d h a	—	—	—	—	I r	—	2 5	—	M y	—	—	—	—	0
B l ea ca npe le	—	—	—	7	I	—	1 3	—	Many	—	0 006	—	—	0
B llea peremp r	—	—	—	—	I r	—	1 3 5	—	Many	—	—	—	—	0
B ll a j m fer n	1 5–3 6 × 10⁻	—	—	10	I r f tet a hed a	—	1 2 5	—	Many	—	0 03	—	300 000	0
B rg ld cal p a	—	—	—	—	—	I l j so dal	—	0 36 × 0 23	—	1	0 03	—	—	0
B k ld d b a	—	—	—	—	—	F l p o la	—	0 45 × 0 25	—	1	0 05	—	—	0
B g d a la l	—	—	—	6–12	—	I l p o da	—	0 45 × 0 30	—	1	0 05	—	—	0
B g ld p	—	—	—	—	—	Fll p o dal	—	0 40 × 0 25	—	1	l o	—	—	0
B k ll l a l	—	—	—	—	—	I l j so dal	—	0 315 × 0 16	—	1	0 04	—	—	0
Be g ld n d	—	—	—	10–20	—	F l j so dal	—	0 345 0 1	—	1	0 01	—	—	0
Sm a r da	—	—	—	—	Irr	—	0 5–4		Many	—	—	—	—	0

[illegible]

brane usually into one rod but sometimes into a bundle of two *Develop*
stages of virus infrequent in polyhedra

(2) *Chemical Composition and Physical Properties* Virus particles suspe[nd]
well in distilled water Virus contains about 14 per cent *nitrogen* 1 3 pe[r]
cent phosphorus 14 per cent desoxyribonucleic acid *no ribonucleic aci[d]*
0 01 per cent iron very little or no lipoids or carbohydrates *Ratio of ade*
nine + thymine to guanine + cytosine 1 34 *Amino acid content of virus (in*
per cent of total nitrogen) cystine + cysteine 0 4 aspartic acid 8 1 *glutamic*
acid 3 7 serine 6 7 glycine 5 8 threonine 6 8 alanine 4 7 *tyrosine 2 4 meth*
onine 1 8 histidine 1 4 lysine 3 0 arginine 18 4 valine 2 4 leucine + isoleu
cine + phenylalanine 7 6 proline 2 3 nucleic acid 16 1 unaccounted for 8 4
Possibly no tryptophan

Virus fairly monodisperse in the ultracentrifuge Sedimentation constant
s_{20} 1871 Svedberg diffusion constant D_{20} 2 15 $\times 10^{-8}$ specific volume 0 ..
Particle weight about 45 6 $\times 10^{-17}$ g or 2 76 $\times 10^{8}$ g /mole

(3) *Immunological Properties* Virus and polyhedral proteins have high
antigenic activity Both are serologically unrelated to host but there may be
some relationship between virus and polyhedral protein

(4) *Susceptibility to Physical and Chemical Agents* Activity of virus is
reduced or lost after freezing boiling drying or treatment with ultrasonics
ethyl alcohol ether alkalis acids and calcium ion In aqueous suspension at
4 C the virus is stable for several weeks Virus particles enclosed within
the polyhedra stored dry or wet retain their activity for many years

(5) *Natural Methods of Transmission* Virus is transmitted from larva to
larva by contaminated food and to the next generation *via* the eggs

(6) *Host Tissue and Cell Tropisms* *Bombyx mori* (L) (Lepidoptera Bom
bycidae) silkworm (FIGURE 1) Virus usually attacks the larval stages seldom
the pupa Virus and polyhedral bodies develop only in cell nuclei of most
organs including blood cells but not in gut cells Transmission to other insect
species not clarified

(7) *Pathology Including Inclusion Body Formation* Virus causes silkworm
jaundice (Synonyms *giallume grasserie Gelbsucht Fettsucht Polyeder Krank
heit*) LD₅ intralymphal about 4 $\times 10^{-13}$ g free virus/larva *per os* about
5 $\times 10^{-5}$ – 1 $\times 10^{-4}$ g or 600 000 1 200 000 polyhedral bodies/larva De
struction of cell nuclei of most organs with formation of enormous numbers of
polyhedral bodies which usually kill the larva or pupa but not the adult
Period from infection to death 6 8 days depending on virus dose and tem
perature Virus can exist in latent form in the host for several generations

Polyhedral bodies are resistant to putrefaction processes and are insoluble in
water and alcohol but dissolve in weak alkalis and acids They are fairly
uniform in size (about 5 μ in diameter) and shape and are crystalline rhombo
dodecahedra They stain after acid or alkaline treatment with fuchsin giemsa
and iron haematoxylin They consist of about 5 per cent virus particles and
about 95 per cent of very homogeneous polyhedral protein Virus particles
are liberated from polyhedral bodies by dissolving the latter (5 mgm /ml)
in 0 006 *M* Na_2CO_3 + 0 05 *M* NaCl The polyhedral protein has a molecular
wieght of 278 000 (s_{20} 12 85 D_{20} 3 12 $\times 10^{-7}$) and splits reversibly by alkali

he dev lopm t l m mbra m rod som w th lend protrus on nd emp y m mb anes ×
1 P lyhed l rus pa t les of *Bomby mor* L pl l d k tages gl id h

treatment to 60 500 (1/6) and irreversibly to 20 230 (1/18) with sedimentation constants of 3 16 and 1 49 Svedberg diffusion constants of 4 80 × 10^{-7} and 6 80 × 10^{-7} respectively The polyhedral protein contains about 15 7 per cent nitrogen 0 06 per cent phosphorus which may not belong to the molecule and 0 005 per cent iron Amino acid content (in per cent of total nitrogen) cystine + cysteine 0 6 aspartic acid 7 6 glutamic acid 8 7 serine 3 0 glycine 3 2 threonine 2 5 alanine 3 4 tyrosine 3 2 methionine 2 3 histidine 4 9 lysine 11 3 arginine 11 0 valine 5 1 leucine + isoleucine + phenylalanine 13 1 proline 2 9 tryptophan 3 2 not accounted for 9 3 per cent

(8) *Symptomatology* No external symptoms of the disease until about two days before death Larvae become swollen the integument becomes opaque in spots shiny in the white race and yellowish in the yellow race The integument breaks very readily releasing a yellowish white liquid of a cream like consistency comprising chiefly polyhedral bodies larvae often still motile at this stage of the disease

Pertinent Literature Aoki and Chigasaki 1921 Bergold 1943 1947 1948 1948a 1950 1952 1952a 1952b 1953 Bergold and Friedrich Freksa 1947 Bergold and Pister 1948 Bolle 1898 Cratia and Paillot 1939 Holoway and Bergold 1953 Paillot 1925 Steinhaus 1949 1949a Yago 1951 Wellington 1952 Wyatt 1952

Bollea efficiens (Holmes) comb nov = *borrelina efficiens* Holmes 1948

(1) *Morphology and Methods of Reproduction* Virus is rod shaped averaging about 350 × 56 5 mμ Morphology and methods of multiplication similar to those of *B bombycis* Many more virus particles in the developing stage than in *B bombycis* Bundles with two rods quite frequent some bundles contain probably four rods

(2) *Chemical Composition and Physical Properties* Ratio of adenine + thymine to guanine + cytosine 0 94 Virus polydisperse in the ultracentrifuge having three different heavy components probably representing the different morphological forms Sedimentation constants s_{20} 1800 2800 and 3640 Svedberg Diffusion constants (mean of 3 components) 2 3 × 10^{-8}, particle weight greater than that of *B bombycis* probably about 116 × 10^{-17} g or 665 × 10^{6} g /mole

(3) *Immunological Properties* Nothing known

(4) *Susceptibility to Physical and Chemical Agents* Virus particles enclosed within the polyhedra stored dry or wet retain their infectivity for many years

(5) *Natural Methods of Transmission* The virus is transmitted from larva to larva by contaminated food and to the next generation *via* the eggs

(6) *Host Tissue and Cell Tropisms* *Lymantria monacha* (L) (Lepidoptera Lymantriidae) *Nonne* nun moth otherwise as *B bombycis*

(7) *Pathology Including Inclusion Body Formation* Virus causes *Wipfelkrankheit* (Synonyms *Wipfelsucht Polyeder Krankheit* nun moth wilt nun moth polyhedrosis) LD_{50} intralymphal and *per os* not accurately determined because of interference by latent disease of test insects otherwise as *Bollea bombycis* Period from infection to death about 13 to 15 days

Polyhedral bodies not regular in size and shape About 2 5 to 10 μ in

diameter irregular tetrahedra or cubes Virus liberated by dissolving the polyhedra (5 mgm/ml) in 0 006 *M* Na_2CO_3 + 0 05 *M* NaCl The homogeneous polyhedral protein has a molecular weight of 336 000 (s_{20} 12 78 D 3 5 × 10) and splits reversibly by alkaline treatment to an unstable component (¼) and irreversibly to 18 270 (1/18) with sedimentation constant of 1 38 Svedberg and diffusion constant of 6 98 × 10^{-7} The polyhedral protein contains about 15 6 per cent nitrogen and 0 04 per cent phosphorus The latter may not belong to the molecule

(8) *Symptomatology* No external symptoms at all until diseased larvae disintegrate liquid flowing out from such larvae is brownish white Diseased larvae migrate to tree tops (*Wipfeln*)

Pertinent Literature Bergold 1943 1947 1948 1952a 1952b 1953 Breindl 1938 Breindl and Jirovec 1936 Escherich and Miyajima 1911 Escherich 1913 Komarek and Breindl 1923 1924 Prell 1926 Roegner Aust 1949 1949a Steinhaus 1949 1949a Wyatt 1952

Bollea reprimens (Holmes) *comb nov* = *Borrelina reprimens* Holmes 1948

(1) *Morphology and Methods of Reproduction* Virus is rod shaped averaging about 364 × 41 mμ Morphology and multiplication very similar to *B bombycis* and *B efficiens* Many virus particles in various developing stages Rods may develop singly or in pairs but usually in bundles of four or possibly more within the developmental membrane

(2) *Chemical Composition and Physical Properties* Virus contains about 15 per cent nitrogen 1 3 per cent phosphorus and about 14 per cent desoxyribonucleic acid Ratio of adenine + thymine to guanine + cytosine 0 71 Virus polydisperse in ultracentrifuge showing three components representing probably the different morphological forms Sedimentation constants 2500 3100 and 4000 Svedberg diffusion constant (mean of 3 components) 1 75 × 10^{-8} specific volume 0 74 particle weight of mature rod about 65 × 10^{-17} g or 393 × 10^{6} g/mole

(3) *Immunological Properties* Nothing known

(4) *Susceptibility to Physical and Chemical Agents* Virus particles enclosed within the polyhedra stored dry or wet retain their infectivity for many years

(5) *Natural Methods of Transmission* The virus is transmitted as *B bombycis* from larva to larva by contaminated food and to the next generation via the egg

(6) *Host Tissue and Cell Tropisms* *Porthetria dispar* (L) (Lepidoptera Lymantriidae) gypsy moth otherwise as *B bombycis* (FIGURE 2)

(7) *Pathology including Inclusion Body Formation* Virus causes polyhedrosis of gypsy moth caterpillar (Synonym Wilt disease gypsy moth wilt *Polyeder Krankheit*) LD_5 intralymphal around 10^{-1} g free virus larva and per os approximately 10^{-4} g or about six million polyhedra/larva but these figures have not been accurately determined because of interference by latent disease of test insects Period from infection to death 10 to 17 days Otherwise as *B bombycis*

Polyhedral bodies of varying sizes from 1 to 10 μ averaging about 3 μ and irregular form with no predominant shape Otherwise as *B bombycis* Virus



Fi ru 2 P lyhed l body f *P th t d p* (L.) d sol t n V ru part les f ri u dev l pm t t g d d t d f th polyhed l body ×50 000

lberated by dissolv n„ the polyhedra (5 m„m ml) n 0 008 M \a (·0 05 M \aCl The homogenous polyhedral pr te n l a a m le ula e gh 2 6000 (s 12 57 D₀ 4 18 × 10⁻⁷) and spl ts revers bly to 4 il revers bly to 15 360 (¹₁₈) v th sed mentat on onstan f 3 t 3 S edberg and d ffus on constants of 6 05 × 10⁻⁷ an l 8 8 × 1 ely The polyhedral prote n conta ns about 1 4 per e t e out 0 045 per cent phosphorus h ch may not belon„ to th m u e

(8) *Syn p oma olog* No ext rnal symptoms at a l unt l ed isintegrate and a bro n sh h te l qu l flo s out

Pe t nent l terature Bergold 1943 194 1948 1948a 1) 1 a l 53 Bre n ll an l J rovec 1936 (laser 1915 (la r an l (l a 1 1 nhaus 1949 1949a Wyatt 1952

B llea olell r a (Ste nhaus) *co nb o* — *Borrel a lell r a* S e nl u 1 4

(1) *Morpl logv a l Mell o ls of Repr d l o* \ ru s ro l l ape μ × 0 mμ Occurr ng in bun lles of se eral members each

(2) (5) I ttle nformat on ava lal le

(6) *Host T ss e a d Cell Trop sn s Prode a praef a* (rote l c l l Phalaen lae (\octu dae)) Western yello str ped army orm

( ) *Pa l logy l d g I cl s o Bo ly Forn al o* \ rus au es p l l e l ss I olyhe lra appear to have four or f e s des hen see n u l n a l are 2 5 μ n d ameter ith an average of about 3 μ General p r pert es prob ally s m lar to those of *B bon b cis*

(8) *Symp omatol gy* Infected lar ae lose appet e l ecome slu sh m ment may turn re ld sh bro n l efore death l dy contents l s ntegrate n o a dark atery mass t l n the nteguments

Pe t nent l terature Ste nl aus 1949 1949a

B l ea campe les (Ste nhaus) *omb* *Borrel* *campeole* Ste nhaus 1949a

(1) *Morpl logv a d Mell o ls of Reprol t o* \ rus rod shape l averag ng app ox mately 300 × 40 mμ Occurr ng n l un lles of se eral meml ers ea l

(2) *Clem al Co pos t o a d I l ys al I ropert es* Rat f a len ne + thy m ne to guan ne + cytos ne 1 35

(3) *Imm ol g cal Propert es* \o information a a lal le

(4) *S sep b l v t I l s cal a l Cle al lge s* Most of tl e phys cal and chem cal agents h cl destroy l acter a also le tr y the v rus In c r p rate l ith n the polyl e lra the v rus reta ns ts nfect ty f r at least a year

(5) *\a ral Mell ds of Tra sn ss o* \ rus tran m tte l from larva t larva b contam nate l food

(6) *Host T ss e a d Cell Trop s ns C l pl l ce e r l eme* B l l (Lep l p e a I er lae) Alfalfa caterp llar

( ) *Patlol g cl l g I l s I lv F rma n* \ rus causes polyl edros s of t e alfalfa caterp llar (Synonyms W lt l sea es lt) I er od from in fe t n t leath al out seven lays P l hedra appear t have 3 6 s les when seen n outl e vary cons derally n hape S ze 1 3 μ than a erage (l 5 μ \ rus l berated l v l sol ng polyhe lra (5 mgm ml) in 0 00(M \a CO_2 +

0.05 *M* NaCl. Most of the general properties of the polyhedra are essentially the same as those described for *B. bombycis*.

(8) *Symptomatology.* Infected larvae show loss of appetite and decreased activity. The normal green color of the larvae changes to a pale yellowish or grayish green; body becomes very flaccid.

Pertinent Literature: Steinhaus 1948, 1949, 1949a; Wyatt 1952.

Bollea peremptor (Steinhaus) *comb. nov.* = *Borrelina peremptor* Steinhaus 1949a

(1) *Morphology and Methods of Reproduction.* Virus rod shaped averaging about 270 × 30 mμ. Occurring in bundles of several members each.

(2) and (3) No information available.

(4) *Susceptibility to Physical and Chemical Agents.* Most physical and chemical agents which destroy bacteria also destroy the virus.

(5) *Natural Methods of Transmission.* Little information available.

(6) *Host Tissue and Cell Tropisms.* *Phryganidia californica* Pack. (Lepidoptera: Dioptidae) California oakworm.

(7) *Pathology including Inclusion Body Formation.* Virus causes polyhedrosis of the California oakworm. Polyhedra appear to have from 3-5 sides when seen in outline and vary considerably in shape. Size ranges from 1.0-3.5 μ in diameter with an average of about 2 μ.

(8) *Symptomatology.* Infected larvae become sluggish in movement and lose their appetite. Natural coloration becomes less intense; recently dead larvae may assume a pink or reddish color.

Pertinent Literature: Steinhaus 1949, 1949a.

Bollea fumiferana spec. nov.

(1) *Morphology and Methods of Reproduction.* Virus is rod shaped averaging about 260 × 28 mμ. Morphology and methods of multiplication similar to those of *Bollea bombycis*. Several virus particles in the developing stage; bundles with two rods quite frequent.

(2) *Chemical Composition and Physical Properties.* Virus particles suspend well in very dilute alkali (about 0.0001 *M* Na_2CO_3) but not in distilled water. Weight ratio of nitrogen to phosphorus 16. The phosphorus of the virus is bound as desoxyribonucleic acid. Ratio of adenine + thymine to guanine + cytosine 0.95. Particle weight about 20.8×10^{-12} g. or 176×10^6 g./mole.

(3) *Immunological Properties.* Nothing known.

(4) *Susceptibility to Physical and Chemical Agents.* Virus particles enclosed within the polyhedra, stored dry or wet, retain their infectivity for many years.

(5) *Natural Methods of Transmission.* Virus is transmitted from larva to larva by contaminated food and to the next generation *via* the eggs.

(6) *Host Tissue and Cell Tropisms.* *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) Spruce Budworm; otherwise as *B. bombycis*.

(7) *Pathology including Inclusion Body Formation.* Virus causing the polyhedral disease of the spruce budworm. LD_{50} intralymphal about 1.5-3.6 × 10^{-9} g. free virus/larva. Period from infection to death about 10 days. Virus can exist in latent form in host. Otherwise as *B. bombycis*.

Polyhedra of varying size, about 1.0-2.5 μ in diameter, and of irregular shape, but tetrahedral forms are frequent. Only about 1.5-2 per cent of their weight

represents the v rus part cles These are l berated by l ol b he | l a (5 mgm /ml) n 0 03 M Na CO + 0 05 M NaCl The e ght ra l to phosphorus of the polyhedral prote n is about 200 but the ph | l ru m not belong to the molecule

(8) *Symptoma ology* No external symptoms unt l abou o l death Lar ae become s ollen and some hat l ghter n colo L l out from d s ntegrated larvae h te to l ght bro n cons t g h l hedral bod es

Pert nent L terature Bergold 1950 1951 1952b 1 3 W l

Genus Bergold a Ste nhaus 1949

Bergold a calypta Ste nhaus 1949 Type Spe

(1) *Morphology a d Me hods of Reprod ct o* V rus s o l l x about 257 mμ long and 41 mμ n d ameter The ro l a e u th n (nt mate) membrane and cons st of spher cal sub un A t us on (about 50 × 10 mμ) extends from one end of the ro k reta n the de elopmental membrane wh ch g es them a le curved appearance V rus develops from a sma l s l re l mental membrane usually nto one rod but occas onall n l V rus de elop ng stages qu te f equent

(2) *Chem cal Compos t o a d Phys cal Propert es* V rus u l welt n d st lled ater Only desoxyr bonucle c ac d s pres aden ne + thym ne to guan ne + cytos ne 1 67 V rus no erv h u n the ultracentr fuge probably because of presence of ar ous morp o g stages Sedimentat on constant 1324 S edberg l ffus on on tan 8 $\times 10^{-8}$ Part cle we ght about 43 5 × 10 g or 2 0 × 10 g mole

(3) *Imm olog cal Propert es* Noth ng kno n

(4) *S s ep b l y to Phys cal a d Chem cal Age s* V rus part les enclose l w h n the capsules stored dry or et reta n the r act v ty for many years

(5) *Na ral Me hods of Tra sm ss o* V rus s transm tte l from larva to larva by contam nated food and to the next generat on v a the eggs

(6) *Host T ss e a d Cell Trop ms Ca oc a mu a a* (Hbn) (Lep l p tera Tortr c dae) (FIGURE 3) The v rus usuall attacks the larval stages and occas onally the pupae De elopment of rus and cap ules usually n cytoplasm of most organs nclud ng bloo l cell th n vacuo es f ary ng s ze (up to 20 μ in d ameter) h ch fuse together The e vacuoles break hen ce l wall ruptures an l enormous numbers of cap su es are l berated into the serum Transm ss on to otl er nsect pec es doubtful

( ) *Pa lology a d g I cl s o I dv I o na o* V u caus ng tl e Kap s us kra kle t (Synonym granul s s capsule l sease) I D a tralymp l al and *per os* not accurately determ ned because of nterference l v latent d sease n test nsects Destruct on of cells of m st organs b f rmat n of enorm us numbers of capsules usuall result ng in death of the pup e I r od f om in fect on to death not accurately determ ne l V rus can ex t n latent f rm f r se eral host generat ons

Capsules are res stant to putrefact on processes an l are n luble n ater and alcohol but l ssol e e k alkal a l ac l They are t very un f rm

0 05 *M* NaCl Most of the general properties of the polyhedra are essentially the same as those described for *B bombycis*

(8) *Symptomatology* Infected larvae show loss of appetite and decreased activity The normal green color of the larvae changes to a pale yellowish or grayish green body becomes very flaccid

Pertinent Literature Steinhaus 1948 1949 1949a Wyatt 1952

Bollea peremptor (Steinhaus) *comb nov* = *Borrelina peremptor* Steinhaus 1949a

(1) *Morphology and Methods of Reproduction* Virus rod shaped averaging about 270 × 30 mμ Occurring in bundles of several members each

(2) and (3) No information available

(4) *Susceptibility to Physical and Chemical Agents* Most physical and chemical agents which destroy bacteria also destroy the virus

(5) *Natural Methods of Transmission* Little information available

(6) *Host Tissue and Cell Tropisms* *Phryganidia californica* Pack (Lepidoptera Dioptidae) California oakworm

(7) *Pathology including Inclusion Body Formation* Virus causes polyhedrosis of the California oakworm Polyhedra appear to have from 3 5 sides when seen in outline and vary considerably in shape Size ranges from 1 0 3 5 μ in diameter with an average of about 2 μ

(8) *Symptomatology* Infected larvae become sluggish in movement and lose their appetite Natural coloration becomes less intense recently dead larvae may assume a pink or reddish color

Pertinent Literature Steinhaus 1949 1949a

Bollea fumiferana spec nov

(1) *Morphology and Methods of Reproduction* Virus is rod shaped averaging about 260 × 28 mμ Morphology and methods of multiplication similar to those of *Bollea bombycis* Several virus particles in the developing stage bundles with two rods quite frequent

(2) *Chemical Composition and Physical Properties* Virus particles suspend well in very dilute alkali (about 0 0001 *M* Na_2CO_3) but not in distilled water Weight ratio of nitrogen to phosphorus 16 The phosphorus of the virus is bound as desoxyribonucleic acid Ratio of adenine + thymine to guanine + cytosine 0 95 Particle weight about 20 8 × 10^{-12} g or 126 × 10^6 g/mole

(3) *Immunological Properties* Nothing known

(4) *Susceptibility to Physical and Chemical Agents* Virus particles enclosed within the polyhedra stored dry or wet retain their infectivity for many years

(5) *Natural Methods of Transmission* Virus is transmitted from larva to larva by contaminated food and to the next generation *via* the eggs

(6) *Host Tissue and Cell Tropisms* *Choristoneura fumiferana* (Clem) (Lepidoptera Tortricidae) Spruce Budworm otherwise as *B bombycis*

(7) *Pathology including Inclusion Body Formation* Virus causing the polyhedral disease of the spruce budworm LD_{50} intralymphal about 1 5 3 6 × 10^{-9} g free virus/larva Period from infection to death about 10 days Virus can exist in latent form in host Otherwise as *B bombycis*

Polyhedra of varying size about 1 0–2 5 μ in diameter and of irregular shape but tetrahedral forms are frequent Only about 1 5–2 per cent of their weight

represents the virus particles These are liberated by dissolving the p (5 mgm /ml) in 0 03 M Na CO_3 + 0 05 M NaCl The weight rati to phosphorus of the polyhedral protein is about 200 but the ph p r not belong to the molecule

(8) *Symptomatology* No external symptoms until about t h death Larvae become swollen and somewhat lighter in color out from disintegrated larvae white to light brown consisting of hedral bodies

Pertinent Literature Bergold 1950 1951 1957b 19 3 Wyatt 1

Genus **Bergoldia** Steinhaus 1949

Bergoldia calypta Steinhaus 1949 Type Species

(1) *Morphology and Methods of Reproduction* Virus is rod shaped averaging about 257 mμ long and 41 mμ in diameter The rods are surrounded by a thin (intimate) membrane and consist of spherical sub units A slender pro trusion (about 50 × 10 mμ) extends from one end of the rod Rods usually retain the developmental membrane which gives them a wider and slightly curved appearance Virus develops from a small sphere within a developmental membrane usually into one rod but occasionally into a bundle of two Virus developing stages quite frequent

(2) *Chemical Composition and Physical Properties* Virus particles suspend well in distilled water Only desoxyribonucleic acid is present with ratio of adenine + thymine to guanine + cytosine 1 6 Virus not very homogeneous in the ultracentrifuge probably because of presence of various morphological stages Sedimentation constant 1324 Svedberg diffusion constant 2 8 × 10^{-8} Particle weight about 43 5 × 10^{-7} g or 250 × 10^6 g mole

(3) *Immunological Properties* Nothing known

(4) *Susceptibility to Physical and Chemical Agents* Virus particles enclosed within the capsules stored dry or wet retain their activity for many years

(5) *Natural Methods of Transmission* Virus is transmitted from larva to larva by contaminated food and to the next generation via the eggs

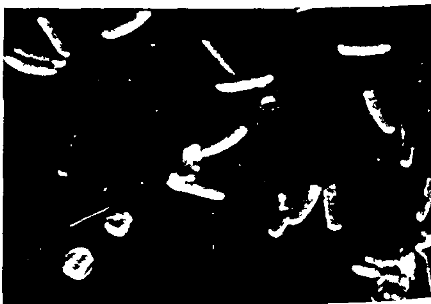
(6) *Host Tissue and Cell Tropisms* *Cacoecia murinana* (Hbn) (Lepidoptera Tortricidae) (FIGURE 3) The virus usually attacks the larval stages and occasionally the pupae Development of virus and capsules usually in cytoplasm of most organs including blood cell within vacuoles of varying size (up to 20 μ in diameter) which fuse together These vacuoles break when cell wall ruptures and enormous numbers of capsules are liberated into the serum Transmission to other insect species doubtful

(7) *Pathology including Inclusion Body Formation* Virus causing the *Kapselvirus Krankheit* (Synonym granulosis capsule disease) LD_{50} intralymphal and *per os* not accurately determined because of interference by latent disease in test insects Destruction of cells of most organs by formation of enormous numbers of capsules usually resulting in death of the pupae Period from infection to death not accurately determined Virus can exist in latent form for several host generations

Capsules are resistant to putrefaction processes and are insoluble in water and alcohol but dissolve in weak alkalis and acids They are not very uniform

in size but usually ellipsoidal with average dimensions of about 360 × 230 mμ They consist chiefly of the homogeneous capsule protein with only about two per cent of virus particles Virus particles liberated from capsules by dissolving the latter (5 mgm ml) in 0 03 *M* Na CO_3 + 0 05 *M* NaCl The capsule protein has a molecular weight of about 300 000 (sedimentation constant 11 8 Svedberg diffusion constant 2 78 × 10^{-6}) and splits by alkaline treatment to components of about 60 000 with sedimentation constant of 3 45 Svedberg

(8) *Symptomatology* No external symptoms of the disease until two days before death when larvae become swollen and somewhat lighter in color The integument breaks readily and a milky white liquid flows out thick with capsules



F[illegible] 3 C[illegible] (Hb) Sph[illegible] ×50 000

Pertinent Literature Bergold 1948a 1950 1952b 1953 Steinhaus 1949 1949a Wyatt 1952

Bergoldia daboia Steinhaus 1949a

(1) *Morphology and Methods of Reproduction* Virus rod shaped averaging approximately 340 × 40 mμ

(2) to (5) Little information available

(6) *Host Tissue and Cell Tropisms* *Peridroma margaritosa* (Haw) (Lepidoptera Noctuidae) Variegated Cutworm Virus and granular inclusions (Synonym capsules) particularly visible in cytoplasm of host cells fat tissue especially affected

( ) *Pa hologv n lud g In l s o Body Forma o* \ ru u n a los s Granular nclus ons ell pso dal th average d mens n u X 2o0 mμ solub e n eak alkal \ ru part c es are l l e te l b granu es 0 0o *M* Na CO + 0 0o *M* NaCl

(8) *Symp oma ologv* T o o three day after nfe on h food than normally rema n maller than normal larvae h langu d appearance but the bod a l rema ns relat el rm

Pert nent L terature Ste nhaus 1949 194)a Ste nhau Hugh \ 194)

Berg ld a la he a Ste nhau 1 4 a

(1) *Morphology a d Me l ods of Reprod o* \ ab ut 300 X 40 mμ

(2) to (5 L ttle nformat on a a lable

(6) *Ho T ssue a d Cell Trop sms Ju o a coe a* Hul Nymphal lae) Buckeye Granu es (capsules eem fo m of hos cells Other se s m lar to *Berg ld a dabo a*

( ) *Pa hology l ud g In l s on Body Forma* \ u lo s Per od f m nfect on to death 6 to 12 da ell pso dal th a erage d mens ons of 4o0 X 30(mμ l sso k k \ irus part cles are l berated bv d ssol ng t e granu es n *M* \

8) *Symp oma ology* D seased lar ae sho v l ttle exte na ease usually change to brown h color an l lose mo t of he m l

Pert nent L terature Ste nhau 1949 1949a Ste nl aus l l 1)49

Bergold a thon p o a S e nl aus 1)49a

(1) *Mo phol gy a d Me l o ls of Repr d o* \ rus d l aped a erag n app ox mately ? 0 X 40 mμ

(2 to (5) L tle nforma on ava al e

(6 *Hos T ss e a l Cell Trop sn Es g e e a raea* (Drury) (Lep doptera A t lae) Salt marsh caterp llar

( ) *Pa hology l l g I l s o Bo l) Form a o* \ rus caus ng granu lo s Granules (capsules) ell p o dal th a erage d mens ons of about 400 X 2 0 mμ Solul e n lo concentrat ons of Na CO

(8) *S n p oma ol gy* D seased lar ae sh no ex ern l s mptoms lea l lar e are flacc l and soft

Pe nent L terature Ste nhaus 1 4)

Be gold a cl storl abd o Wasse an l S e nhaus 1951

(1) *Morpl ology and Me l ods of Reprod n* \ rus rod shaped a era ng app ox mately 2o0 X o0 mμ Morphol an l mult pl cat o s m lar t those of *Bergold a calyp a* De elop ng stages an l en p t le elop mental meml ranes present

(2) to (5) L ttle nformat on ava able

(6) *Ho T ssue and Cell Trop sms Arg r a a tel a a* (Walker) (Lep doptera Tortr c lae) Red banded leaf ro er

(7) *Pathology including Inclusion Body Formation* Virus causing granulosis granules (capsules) ellipsoidal with average dimensions of 315 × 160 mμ dissolve in weak alkali Virus particles are liberated by dissolving the granules in 0 04 *M* Na_2CO_3 + 0 05 *M* NaCl

(8) *Symptomatology* The green and yellow green of the healthy larvae changes to a straw color to whitish in diseased larvae

Pertinent Literature Wasser and Steinhaus 1951

Bergoldia nosodes Hughes and Thompson 1951

(1) *Morphology and Methods of Reproduction* Virus rod shaped averagin approximately 275 × 65 mμ *Morphology and multiplication similar to* those of *Bergoldia calypta* and *Bergoldia clistorhabdion* Developing stages and empty developmental membranes present

(2) to (5) Little information available

(6) *Host Tissue and Cell Tropisms* *Sabulodes caberata* Guenee (Lepidoptera Geometridae) Omnivorous Looper Virus attacks paticularly the fat tissue granules appear to form in the nucleus and not in the cytoplasm as they apparently do in the type species *Bergoldia calypta*

(7) *Pathology including Inclusion Body Formation* Virus causing granulosis Period from infection to death varies considerably with most of the *mortality occurring in 10 to 20 days after infection* Granules (capsules) ellipsoidal with average dimensions of 345 × 170 mμ Virus particles are liberated by dissolving the granules in 0 1 *M* Na_2CO_3 + 0 05 *M* NaCl

(8) *Symptomatology* Diseased larvae become somewhat mottled in appearance The green integument becomes blotched with white and a chalky white area appears along the ventral side

Pertinent Literature Hughes and Thompson 1951

Genus Smithia *gen nov*

Smithia rotunda spec nov Type Species

(1) *Morphology and Methods of Reproduction* Virus spherical with an average diameter of about 65 mμ * internal structure discernible some virus particles have a thin straight tail or protrusion about 70 mμ long and 10 mμ in diameter similar to that of the virus rods in the genera *Bollea* and *Bergoldia* Such spherical particles with a tail are similar in appearance to the tadpole shaped bacteriophages Frequently four or five spheres are contiguous forming a beaded rod occasionally denser rods are seen of about the length of four to six spheres lined up in a row These rods seem *to disintegrate* into spherical virus particles Empty tube shaped and filamentous membranes can be found

(2) to (4) Little information available

(5) *Natural Methods of Transmission* The virus is transmitted from larva to larva by contaminated food and from generation to generation *via* the eggs

(6) *Host Tissue and Cell Tropism* *Arctia villica* L (Lepidoptera Arctiidae)

M m ts t k f m lect m graph p pa d by th th f m polyh d al bod f 4 1
ll a L k dly ppl d by D K th M Sm th

Cream spotted Tiger moth (FIGURE 4) Development of vir ı l ı bodies only in cell nuclei of the host larvae as in the genu /
(ı) *Pathology including Inclusion Body Formation* \ rı ıu hedrosis Destruction of cell nuclei with formation of c r ı polyhedra Virus can exist in latent form in the host l J of various shapes and range from about 0 5 to 4 μ in diamet r \ are liberated by dissolving the polyhedra in weak alkal I l ferently from the polyhedra of the genus *Bollea* They l r pieces which show very characteristic spherical cavities tr n

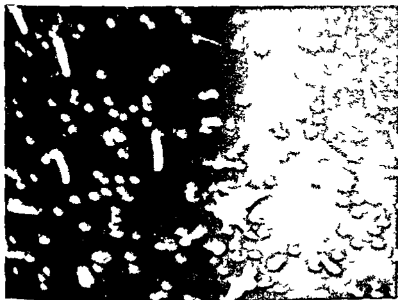


Fig 4 Virus particles ... (L) ... Section ...

ical virus particles had slipped out Occasionally the imprint of a tadpole l rm can be seen Section through the polyhedra show a characteristi pitted structure

(8) *Symptomatology* No information available
Pertinent Literature Smith (1951) Smith and Wyckoff (19 0 1) 1)

In addition to the above mentioned viruses there are many more known from Lepidoptera and some Hymenoptera (Bird 1951) Steinhaus (1949) estimates that about 100 polyhedral di eases are known at present Several of these viruses have been isolated and described but no names have been published There are also some doubtful cases of virus diseases described in Diptera (Rennie 1923 Weiser 1948) and Orthoptera (Gregoire 19 1) which

need more investigation Since the technique of isolation of certain insect viruses is well established it is to be expected that many more will be described in the near future It is also likely that new genera of insect viruses will be established

The importance of a virus type culture collection for an efficient nomenclatural system was emphasized at the Conference on Viral and Rickettsial Classification and Nomenclature in New York January 1952 Insect viruses are an ideal subject for that purpose since the virus particles do not lose their infectivity for many years when they are stored enclosed within the inclusion bodies

In conclusion the author would like to mention a proposal which was discussed by the virus sub committee in Rio concerning the formation of a committee to be consulted by an author and which would share in the responsibility of naming and classifying new viruses It may go too far to formulate this as a rule Yet authors should be invited to discuss such problems before publication with the members of an international committee of three to five experts in their field At the Conference on which this monograph is based Mayr (p 391) suggested a similar plan by proposing that virologists would avoid many of the nomenclatural difficulties of the zoologists if they would assign the authorship of their scientific names to national or international committees and charge them with the responsibility of issuing a list of official names The author is very much in favor of any such attempt and hopes that all virologists will take the excellent advice of Ernst Mayr very seriously In many cases it would be much easier and more satisfactory to solve a problem in that way before rather than after publication Such cooperation would help to prevent the present confusion in the classification of such groups as bacteria fungi and insects

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TAXONOMY OF INSECT VIRUSES

By Edward A Steinhaus

Laboratory of Insect Pathology Division of Biological Control College of Agriculture University of California Berkeley California

Although there is evidence that viruses unassociated with demonstrable inclusion bodies do cause infection in insects the best known insect viruses today are those characterized by the presence of either polyhedral or granular inclusions in the infected cells of the host These viruses constitute a group of agents in many ways distinct from most other animal viruses and from plant viruses Their location within the polyhedral or the granule like inclusion, the occurrence of many of them in uniform bundles of several virus particles each or regularly as individual rods and their relatively large size (generally about 40 millimicrons in width and from 2 0 to 360 millimicrons in length), aid in distinguishing them from most other viruses This distinctiveness and the rapid progress being made in demonstrating them has enhanced the need and desire to arrange them according to some scientifically valid yet convenient system of nomenclature and classification

Acknowledging earlier contributions made toward the nomenclature and classification of insect viruses I attempted to take a step in the direction of obtaining a satisfactory taxonomic arrangement of these agents with the publication in 1949 of certain provisional concepts along this line The foremost general classification of viruses at that time as now was that presented by Holmes (1948) in the sixth edition of Bergey s *Manual of Determinative Bacteriology* In my 1949 paper certain errors weaknesses and inadequacies in the Holmes treatment of the insect viruses were recognized and pointed out but I did acknowledge and accept the thesis that the binomial system of nomenclature could be applied to properly described insect viruses as well as it could to rickettsiae bacteria and other microorganisms Despite initial and continuing mild disagreement in some quarters I remain convinced of the validity of this position

Basic difficulties with regard to virus nomenclature have been greatly ameliorated since the recent adoption of the International Bacteriological Code of Nomenclature There appears to be general agreement that the Code as stated therein legitimately concerns viruses as well as bacteria and related organisms Most of the difficulties at least as far as the insect viruses are concerned, lie with matters of classification and phylogenetic arrangement A beginning toward solving the problems of classification has been made however and we have every reason to believe that by the gradual processes of evolution rather than by edict we can attain the taxonomic goals we seek The tendency of many bacteriologists and virologists especially those who disdain systematics to demand a rigid completed and final classification and list of names of the agents with which they work reflects a lack of appreciation for the slow gradual revisional process by which such information and knowledge is evolved and established. Also to be guarded against is any tendency on the part of international organizations and committees (as valuable as these bodies may

be for refereeing and for providing guidance in taxonomic procedures) arbitrarily to delay or prevent individual initiative and thoughtful contributions in virus systematics* by instituting blanket *restrictions without regard to possible exceptional areas where real advance may be made* Nor should scientific journals be influenced by such bodies to refuse publication of adequately and properly described species of viruses It is because workers at our laboratory have felt that many insect viruses can be adequately treated from a taxonomic standpoint that we have described new genera and species of these agents In no sense have our proposals been presented with the presumption that they are final or that our methods and conclusions should be taken as generalizations *applying to animal and plant viruses as a whole*

In virus taxonomy as in all other branches of systematics we must avoid the common error of confusing nomenclature (the naming of organisms), classification (the ranking and orderly arrangement of organisms) and identification (determining the identity of given organisms with described organisms) While closely related these disciplines are nevertheless separate and distinct That nomenclature without classification is possible cannot be denied That nomenclature within groups of viruses that cannot be adequately described or defined taxonomically is wise and scientifically sound is another matter The naming *of viruses and their arrangement into taxonomic groups of phylogenetic significance* constitute areas of activity and scholarly consideration quite apart from that of identifying viruses The proper identification of an organism can require as much skill and be as difficult a procedure as ascertaining its proper taxonomic position in a system of classification Unfortunately as regards the bacteria for example it is frequently easier to describe the organism as a new species than to identify it with one already described Let us hope that the same practice will not prevail in the case of viruses

We must also keep in mind the ultimate objective or purpose in naming and classifying living organisms To most authorities this objective is to provide internationally recognized names for organisms and to reveal the phylogeny ancestry or interrelation of species in such a manner as to gain a greater understanding of any particular organism that concerns us With this understanding as a basis it is possible to make valid taxonomic and biological generalizations concerning closely related organisms Knowing into which group an organism naturally falls and the basis of its similarity and relation to other organisms enables us to comprehend or predict its own properties characteristics inherent nature and potentialities more readily and more thoroughly Not to recognize these transcending goals of systematics is to risk the acceptance of an unsatisfactory and faulty taxonomic structure built on a foundation of impulsive expediency

As is to be expected newer knowledge concerning the insect viruses has been

Th s m ght oste s bly be don th grou ds f w t g unt l m re f ct are [illegible] ilable Wh l t is un d bt dly tru that w n t p *perly clas fy cc rd g to phyl g y* ruses bo t wh h w k ow l ttle thi g t l tru th t th l e f as n g e be carried too f A tatement from Geo g G *ylord* Simpso book Th Mea ing f E l t n (Y l U ers ty Pres 1949 p 272) p pos her On co d g th m facts d s pe d j dgm t to what m a g th y m ght event lly h This s unds lik fi ld and it h s ev been h led (f ll w ng B on wh ev s eed d maki g dis ery by h famed i d t m thod) as th prope p ocedure l s i In re lity g be g facts w thout f rm lated reas for d g and a pretty good id as t wh t th f ts m y m t l oc pati and h s ot been the m thod f y mpo ta t c t fi d

brought forth since the time my principal proposals were advanced The entire field of virus systematics has received new and critical attention from many quarters and it might be added from systematists and nonsystematists alike It behooves us therefore to examine here the taxonomic status of the insect viruses in light of these new developments Of particular concern is the matter of the criteria to be used in determining the limits of the various taxonomic categories (particularly genus and species) into which the insect viruses fall It may be emphasized that we are concerned here only with those viruses that cause infection and disease exclusively in insects Those viruses that cause disease in other animals or in plants and are transmitted by insects are not included or referred to in this discussion

Criteria for Species

It would be unnecessarily redundant to review here the numerous well known arguments concerning what constitutes a species of virus Instead let us briefly consider the matter of ascertaining whether or not what we know about insect viruses enables us to gain some sort of a concept as to what constitutes a species within this particular group of agents

At present there are 15 named species of insect viruses Approximately one hundred *apparently distinct diseases of insects have been described as caused* by viral agents Whether or not each of these diseases is caused by a distinct species of virus is not known but in any event it is probable that a considerable number of separate viruses is involved To judge from those insect viruses that have been isolated and described it is apparent that it is not easy to provide a definition of a species that will be applicable to all cases or that will please everyone Without the tool of cross fertility which is available to taxonomists studying sexually differentiated organisms it is obvious that at the present time we are forced to rely on other less direct methods of delimiting a species The idea that a species of virus should consist of a group of individuals having many characters in common and differing from all other forms of life in one or more significant ways would I imagine be generally accepted The difficulty arises in attempting to decide just how much difference must exist between two viruses before one is justified in considering them as two distinct pecies In spite of the difficulty in arriving at a generally acceptable definition of a species and remembering that the category is a taxonomic concept and not a concrete entity it is becoming increasingly clear that at least apparent or so-called species or other orderly groups need to be established if any progress toward a logical classification of the viruses is to be attained

Whatever the nature of the characters or criteria used for classifying viruses they ideally should be characters or properties of the viruses themselves It is to be hoped that some day the equivalent of the aim of modern taxonomy to consider characters determined by one or a definite number of genes acting in a known manner may in effect be realized for viruses Such attributes as those having to do with symptomatology pathology and even inclusion body formation while of possible great value in the identification of viruses through the differentiation of the diseases they cause are not strictly characters of

viruses and hence cannot with accurate logic be used in a major way to classify the viruses themselves according to species In other words it is the viruses not the diseases that we are endeavoring to clas ify This should not mean, however that descriptions of virus species or genera need to be devoid of any reference to significant symptomatological or pathological details which may accompany the usual activity of the virus Most insects uffering from polyhedroses, for example show symptoms which, in general, are different from those seen in insects suffering from granuloses Knowledge of these differences makes a guess as to the generic identification (if one accepts the present classification) of the causative virus much more reliable and worth while This is because most of the polyhedroses exhibit symptoms fairly uniform in appearance and fairly distinct from most other virus infections A similar situation exists among the granuloses Clearly, it seems to me such criteria as those concerned with morphology, methods of reproduction chemical compos tion physical properties serological, and immunological properties and susceptibility to physical and chemical agents—in other words the leading criteria agreed on by the Virus Subcommittee of the International Committee on Bacterial Nomenclature (Andrewes, 1951)—should constitute the basis of a classification of viruses

When it comes to classifying the insect viruses as to species, we meet head-on many of the same problems confronting specialists in other groups of viruses In the case of those viruses causing polyhedroses and granuloses, the morphol ogy of the individual virus rods is ordinarily so uniformly similar that this criterion is of little value in differentiating species within each of the two ge era concerned It presents a situation similar to that found in the bacterial family Enterobacteriaceae in which the morphological differences are usually not great enough to be of material aid in differentiating species In the case of the polyhedrosis viruses some slight help may be found in distinguishing species by the number of individual virus rods characteristically found in each bundle It is possible that further taxonomic value may be realized in certain forms observed by Bergold as well as by ourselves and which Bergold (19-0) interp ets as 'probably stages of multiplication"

For some of the insect viruses we have a fair knowledge of their chemical composition and physical properties as well as meager information concerning their sensitivity to physical and chemical agents Unfortunately, this informa tion is proving of limited value in separating species inasmuch as there have been revealed a et onl minor variations in these qualities since they are associated with the different insect viruses Very probably one of the safes criteria to use in differentiating these viruses will be that based on serological and immunological differences Very little work has been done toward deter mining the antigenic makeup of insect viruses from a comparative standpoint The rewards of such a study undoubtedly will be great and significant

A matters tand therefore, the primary criterion that by itself has been fairly reliable in differentiating the insect viru es at what may be considered the pecies level is a physiological one namely pathogenicity or, more ac curately host specificity It is largely on this basis that the species in each of the four presently recognized genera of insect viruses are distinguished Nat

urally such a criterion has its limitations and can be used only in so far as it represent a stable quality of the virus. It was precisely for this reason that of the numerous viruses (as represented by recorded virus infections) known to infect insects the writer, in 1949 recognized or described and named only six polyhedrosis viruses and five granulosis viruses. Most of these viruses as well as certain viruses described since then are known to have distinct patterns of pathogenicity as determined by cross infectivity tests and different host specificities. The relatively high degree of host specificity shown by the known insect viruses is a valuable taxonomic attribute but there is no certainty whatever that all insect viruses will show this marked specificity (indeed there are indications that at least some insect viruses can bring about infection in insects other than their natural host) or that variants having wider host ranges will not appear. For those species that we have recognized however the specificity, as far as the different insect hosts are concerned appears to be sufficiently strong to give it the stability required. Obviously it will be impracticable to run infectivity tests with each potentially new species of virus in all the hosts of already described species and to test all the previously described viruses in the host in which each new virus is discovered. There appears to be no reason to doubt that most of the insect viruses presently recognized as valid species are in fact distinct. Before many more new species (particularly among the polyhedrosis and the granulosis viruses) are described however criteria in addition to host specificity and applicable at the species level will have to be used.

Criteria for Genera

Since probably it is generally agreed that a genus is essentially a group of related species separated from other such groups by a distinct gap,* the first difficulty we meet in deciding on the boundaries of a genus of viruses concerns the magnitude of the differences required to separate one group of species (i.e. one genus) from another. Furthermore just how closely related must the different species be to justify their inclusion in the same genus? It is to be understood of course that the recognition of monotypic genera requires extrapolation from polytypic genera.

In the classification of insect viruses advanced by the author in 1949 four genera were recognized largely on the basis of the morphology and nature of inclusion bodies formed when the latter were present. The genus *Borrelina* Paillot (See PLATES 1 and 2, FIGURES 1–12) included those viruses causing insect diseases characterized by the presence of more or less uniformly shaped polyhedral inclusions in the infected cells of the host. A virus associated with polymorphic inclusions (annular, spherical, globoid and elongated structures) of very irregular shape and size and found in the cytoplasm of the infected cells of the host was placed in the genus *Paillotella* Steinhaus, so far a monotypic genus. The genus *Bergoldia* Steinhaus (See PLATES 3 and 4, FIGURES 13–24) was represented by those viruses causing insect diseases characterized by the

* Mayr (1942) has offered the following generic definition: [illegible] species [illegible] group [illegible] of presumably [illegible] phylogenetic origin [illegible] separated by [illegible] decided [illegible] from other [illegible] group [illegible] be postulated for practical reasons that [illegible] shall be in [illegible] the [illegible] of the group.

presence in large numbers of very small but microscopically visible granular inclusions in the infected cells of the host. A virus claimed to be the cause of a disease in the larva of the honeybee in which no visible pathological inclusion body of any kind is produced was retained in the genus *Morator* Holmes where it was placed by Holmes in 1948. This is also a monotypic genus. The descriptions of these genera as presented in 1949 (Steinhaus, 1949b) were intentionally made broad and extensive to permit the inclusion of the inevitable subgeneric differences and variations to be found in future new species. It was felt that not enough concerning the characteristics of the members of the different groups of insect viruses was known at that time to generalize at the generic level. When considered *in toto* however each of the descriptions was made adequate to distinguish the various generic groups. Revision and augmentation of the descriptions were expected as new knowledge was forthcoming.

Certain features of this arrangement of genera are not entirely satisfactory from the viewpoint of ideal systematics. In the first place as already stated in a previous paragraph the separation of the genera *Borrelina*, *Bergoldia* and *Paillotella* largely according to the type of inclusion body produced does not strictly speaking constitute a separation according to distinct characters of the viruses themselves. True, the different kinds of inclusion bodies probably represent and are a reflection of basic differences in virus activity and the three types of inclusion bodies so far recognized do appear to represent three naturally distinct groups. Nevertheless it is a grouping based on entities that are not in themselves infectious agents (*i.e.* viruses). Furthermore apparently not all insect viruses are associated with recognizable inclusion bodies. Holmes has made one of these which was claimed to cause sacbrood in the honeybee the type and only species of the genus *Morator*. It is not to be expected that all noninclusion viruses will be found to be so uniformly similar as to warrant their inclusion in a single genus. There will probably have to be several genera of insect viruses not associated with inclusions. How these genera would be distinguished remains to be seen. The significant thing is that all genera could not be separated on the basis of the morphology and nature of inclusion bodies. This situation however should not present insurmountable difficulties if it is remembered that values of taxonomic characters need not be constant from group to group. A given character in one group does not necessarily have the same value in another group. Furthermore the *possibility should not be ruled out that some of the* noninclusion viruses causing diseases in insects may be more closely related to certain viruses outside the family Borrelinaceae (*i.e.* to viruses not affecting insects) than to other insect viruses.

A further disquieting feature of the present generic classification of the insect viruses has to do with the validity of the genera *Paillotella* and *Morator*. In neither instance has the type species of these monotypic genera been purified and its morphological properties accurately determined. *Paillotella pieris* (Paillot) Steinhaus, the type and only species of the genus *Paillotella* was originally named *Borrelina pieris* by Paillot in 1926. Apparently Paillot using dark field microscopy saw it in larvae of the cabbage butterfly of Europe *Pieris brassicae* (Linn.). *Morator aetatulae* Holmes, the cause of sacbrood in

the honeybee *Apis mellifera* Linn ar l cons dered the type pec es of the genus *Morator* was so named by Holmes in 1948 In p r ious publ cations I have recognized these viruses pr mari y because of t e r importance in representing obviou ly different virus types and to pro k f r a nt nuat on of the previ us y proposed names unt l wiser dec n al l mak relating to the pos t on or to the valid ty of the spe e r I Th s recogn tion was offered merely as a temporary expe lent I I f lt hat aut on should be exer sed n uppres ng the names an l th at t u he s mewhat presumptu ous to do as matters then stood Ina n I h I am d neither species I felt that their temporary retent r l l m tatement of 1949 to the effect that ne pecies of in e t n an e l nly after the virus itself has been demonstrated a n all t nct entity by accepted phys cal or v sual means (In l t r s m l r reasons that *Borrel a brassicae* Pa llot as ret ne l a l ar F llot) In our laboratory we have ass gned ne ra p e h ch at least have been demonstrated with the ele r an l ose p proximate size an l shape have been include l n t p t

Whether or not the posit n just l *Pa ll lla* and *Morator*) rema ns a justifabl one is I r ument A matters stand at present it does not ap ear r a t w at exactly could be accompl shed by supp re n th u u h a t n at l ast in the case of *Morat r ae at l* er n re qu r ng the demonstration of the m rph L gr rg t a name The on y pos ble value n reta r ng l r l l ty the spe cies in eac of the t o genera l e ng e t ru l ifferent from the other and from those n e *l rrel a* and *I erg ld a* The virus nature of *Pa llotel a p er* wa e idence l by the presence of small part les f le r l an Pa ll t 1924 1926a 1926b) described as be n a so l r re These parti cles were virtually as well demon tr te l m l p rt l s seen by Pa llot in the case of s lk orm u l l l *l rrel a bombycis* (the type spec es of the genus *Borr l a* It la e l see Hughes 19 0 that the small part cles seen n l sease l lk n l P a e t but questi ned by Glaser and Cou dry 1928) are le s l l l u te v ru and har acterist c of known polyhedr s r e That I n te f the ize of these part cles d fers from th e h an l el e tr m r s n t sur pris ng m v ev of the improbabl t f l t n g re s all urate measure ments of very small part les l y rk r ll n r l the meth d used l y Pa ot Thus the spe th name propose l by h m ppear t have a quired ad it onal val d ty Furthermore n e Pa ll t escr bed his *B rrel a b mby cis* an l *Borrel a p eris* n about the same t n t *l rre na b mbyc s* had been retained for the s lk rm virus there appeared to be s me just f ca tion for retain ng the spec f ep thet *p er s* f r the cabb ge butterfly virus Because of ts extremely polym rph sm la son l les an l the r manner of formation however it was ob ious that *Pa ll te la p er s* l d not bel ng to the genus *B rrel a* (where Pa llot an l H lm s l ad p la ed t h h haracterized by the presence of typ cal poly edral nclu n an l that t had to be rem oved

from the genus *Borrelina* Since there was no previously established genus to which it could be transferred, it became necessary to propose a new one *Paillotella* for which it would become the type species by monotypy Thus this cabbage butterfly virus inadequately described by Paillot and still awaiting further elucidation, became *Paillotella pieris* (Paillot) Steinhaus (1949a)

In a sense, there is an alternative to either completely accepting the two genera in question or completely abandoning them That is the two genera and their type species might tentatively be assigned to a *Genera et Species Incertae Sedis* group or possibly to an even larger assemblage including not only insect viruses but uncertain species from all groups of animal viruses Such a designation as that of *Imectoriae* as proposed by Limasset (1948) or that of *Imperfectly Known Viruses* as proposed by Bitancourt at this Monograph (page 448) might be considered At least, this might be the temporary fate of *Paillotella pieris* and it is hereby suggested that this species be retained for the time being under the designation *Genus et Species Incertae Sedis* In the absence of clear cut data concerning the morphology of *Morator aetatulae* however it appears justifiable to suppress the name Should the sacbrood virus ever be morphologically demonstrated and properly described there probably would be no reason why at least the specific epithet proposed by Holmes could not be resurrected It must be pointed out however that an insect virus not associated with inclusion bodies has recently been isolated (Steinhaus 1951, Wasser 1952), and that this virus has been placed tentatively in the genus *Morator* Wasser (1952) has proposed for it the name *Morator nudus* In light of the dearth of systematic data pertaining to *Morator aetatulae* the genus could be reconstituted with *Morator nudus* as the type species

We can be reasonably but not absolutely certain of the validity and stability of the genera *Borrelina* and *Bergoldia* as distinguished by their type species The question may be raised as to just how inclusive these genera are to be Whether or not a granulosis virus may occur in polyhedral inclusions or *vice versa* is not known A final answer to this question probably awaits more information concerning the genesis of the two types of inclusion bodies

Smith and Wyckoff (1950 1951) suggested that some of the polyhedrosis viruses are spherical in shape If their observations are confirmed or expanded certain taxonomic revisions will in all probability be required if morphological characteristics of the virus are to be given the taxonomic importance one might expect If the spherical forms are not placed in a new genus the possibility of the genus *Borrelina* being divided into two subgenera should be considered * The taxonomic position of the viruslike bodies observed in the blood of the house cricket by Gregoire (1951) is likewise uncertain in spite of some resemblance to the granulosis viruses A similar taxonomic uncertainty concerns the agent described as a virus by Dutky and Gooden (1950) who discovered it in diseased grubs of the Japanese beetle *Popillia japonica* Newm In our laboratory electron micrographs of infectious material kindly supplied by Dr Dutky,

* At th C f o wh h this monog ph is b ed Dr B rg ld propos d th ew g us *Smithia* to cl d sph c l rus r f rr d t th Sm th d Wy k ff r f s t d d fou d polyh d f *A t ll* (Lin) Acc d g t D B g ld od-sh ped ru p t l s oc as lly oc m g th ph l p t cles If f rth t dy hould how a los r l t on b p f thes p t les to those in th g *B rrel na* t p bl that *Sm th* m y be m de bg

showed the presence of bodies approximating rickettsiae in size and superficially at least bearing no clear relation to any of the known insect viruses Similar observations have been made recently by Willis and Martignoni (1952) in the case of diseased *Melolontha vulgaris* F

Higher Categories

The possibility at this time of establishing basic criteria for categories of insect viruses above the generic level appears to be a rather remote one At least this seems to be the case if we adhere to the principle of classifying viruses on the basis of their own characteristics rather than on manifestations of their activity *The present expedient places the insect viruses in the family* Borrelinaceae suborder Zoophaginеae on the basis of the type of host they infect According to Holmes s system the suborder Zoophagineae includes those viruses causing infection in animals To the family Borrelinaceae is assigned those animal viruses inducing diseases in insects as exclusive hosts To be sure such host selectivity may represent or be a reflection of basic physiological properties of the virus but it probably is not the type of characterization that we would use if we knew all there is to know about the origin evolution and phylogenetic relationships of viruses

It must be remembered however that unless we insist on the unreasonable goal of a final and perfect classification we must be willing to accept from time to time rational expediencies and logical arrangements even though provisional if we are to obtain any practical usefulness or convenience from the classification we construct Accordingly until genuine phylogenetic relationships between insect viruses and other viruses are shown to exist at the family level or below I see no valid reason for not provisionally accepting the familial designation Borrelinaceae to include the insect viruses It would appear that at least the polyhedrosis and the granulosis viruses fall within the broad boundary of what probably constitutes a family Whether or not the limits of this family should be extended to include viruses affecting arthropods other than Hexapoda will have to be considered if and when viruses are found in such animals Holmes (1948) includes the word arthropods in his characterization of the family Viruses causing polyhedral wilt and other diseases in arthropods In his key to the family however his characterization reads Inducing diseases in insects as exclusive hosts Perhaps the entire characterization of the family Borrelinaceae as given by Holmes may for the time being and on the basis of present knowledge be restated simply as Viruses causing infections in insects The phrase and other arthropods could be added at a later date if viruses that belong in the family Borrelinaceae are found in these animals

Further Phylogenetic Considerations

One feature pertaining to the insect viruses that has intrigued workers in this field is the limitation of known infections by these agents almost exclusively to those insects in the orders Lepidoptera Hymenoptera and in a very few instances Diptera Almost 90 per cent of the polyhedroses and granuloses

occur in Lepidoptera Although the phylogenetic relationships between the various insect viruses are only inadequately known it is of interest to examine the phylogenetic relationships of their insect hosts for whatever light these may cast in the direction of the eventual classification of these agents For example it is thought provoking to realize that the *phylogenetic gap between* the noctuids (Lepidoptera) and the sawflies (Hymenoptera) both of which are subject to virus infections is considerably greater than that between the susceptible noctuids and the apparently insusceptible phycitids (both Lepidoptera) This and other similar examples indicate that phylogeny of the host species is not the entire common denominator to the phylogeny of the viruses Cursory examination points to at least one other possible factor that having to do with the insect s nutritional ecology and the probable relation this has to the host to-host transmission of the virus It is probably no accident that the great majority of those insects subject to polyhedroses and granuloses like the noctuids feed on the surface of leaves and foliage Very few virus diseases are known among those insects which feed within plants or like many of the phycitids on stored food products A polyhedrosis has been reported (by Lotmar 1941) in the larva of the webbing clothes moth *Tineola bisselliella* (Hum) That all the so-called polyhedra this worker observed in this insect actually represent a virus infection needs confirmation Attempts by Smith and Wyckoff (1951) to demonstrate virus particles within certain of these bodies were unsuccessful Furthermore contrary to the situation with any other known polyhedrosis in which the inclusions are formed within the nuclei of the infected host cells Lotmar reports that the polyhedra also occur in the cytoplasm of midgut epithelial cells the nuclei of which remain normal That many sided inclusions not containing virus particles may occur in the cytoplasm of cells of apparently normal healthy larvae is indicated by the finding of polyhedralike inclusions in the cytoplasm of midgut epithelial cells of healthy larvae of the variegated cutworm *Peridroma margaritosa* (Haw) by Mauro Martignoni working in our laboratory Dr Lotmar kindly sent our laboratory representative slides of sections of the diseased *Tineola* larvae Examination of these slides showed polyhedral bodies in the nuclei of cells of the adipose tissue entirely similar to those characteristic of polyhedroses Slides showing the polyhedralike bodies in the cytoplasm of the cells of the gut epithelium were not so convincing It is possible that they represent secretory deposits or some other nonvirus phenomenon Further observations and experimentation including infectivity tests need to be made before the matter of intracytoplasmic polyhedral inclusions can be clarified

In any event with an exception or two it would appear that typical polyhedroses and granuloses occur primarily in those insects that are open leaf feeders while closely related insects that do not have this type of feeding habit generally are not known to suffer from these types of virus infections Why such diseases have not been reported from all well studied leaf feeders in the orders Lepidoptera and Hymenoptera can only be guessed Undoubtedly many more examples of these types of virus infections remain to be discovered in these insects The fact that none of these diseases have been found in such coleopterous leaf feeders as the chrysomelids is also interesting In all prob

abil ty the nutritional factor (ar l the accompanying fact r of easy transmi sion in those insects feeding on open leaves) is nly one of a complex of factors that might determine a host s susceptibility or insus cp t bil ty

In this connection it woul l be of interest to leterm ne the relative frequency with which a particular virus disease occurs in a p r cular in ect (with a wide food or host plant range) feeding on each of ts l st pla ts In other words will a polyhedrosis or a granulosis be foun l t qual frequen y in insects of a part cular species feeding on each of its ma l st plants or does the disease occur more frequently when the insect fee l p r i ular ne of its host plants?

Re apitul i

In the foregoing discuss on an attem h le made 1) to clarify the objectives of nomenclature and clas t pertain t the inse t viruses (2) to explain further the bas s f t m p pr posals concerning insect viruses made by the author in 194? at re-examine the criteria that appear to be valid in establish a sat t r l st ati n of the insect viruses

It would appear that the ultimate l c t purpose to be sought in classifying the insect viruses should be t l e l la sical taxonomy in general namely to ascertain the p hyl p st rr lati n t pecies in such a way as to obtain a greater un ler t in l r p of und kno vledge of them an l their place in nature By k o t l h tax n m group a particular virus naturally falls and its r l t ther ruses it n pr per t es characteristics inherent nature an l t t n be m re easily and more thoroughly comprehended an l ge ral t o as be m le more readily

In 1949 I proposed largely out f re e t a rev f the lass f cati n of the insect viruses as had l een presc te l l s H me f p t tnat t me the grouping of the insect viruses was n t at r c n r ne n keep ng with the facts as they were known l y pe p le a tu lly rk n with these agents This revision was suggeste l un ler n illu i n that t represe ted in any sense a final concept Instea l the proposal was merely an att mpt to bring the nomen lature an l classif cati n f th s group f agents n line with what was kno n of their properties an l relationsh p s It was expe ted that numerous further advancements along these lines w ul l be m le as ur knowledge f iruses in reased Preference was indi ate l for the u e f the l in mial system of nomenclature for the insect viru es an l th still remains a c n ct on of the auth r

The criteria l erein proposed for clas fying the insect viruses are not g r f cantly different from th o l p ted l y the v rus subcomm ttee of the inter nati nal committee on ba terial n menclature What appear to be the most val l criteria nclu le morph l gy meth ds f repr duction hemical composit on physical properties ser l g al and imm unological properties su ep t l d ty to physical and chem cal agents an l to a l m ted extent l fferential h st susceptibility Such qual t es as ymptoms and patholog cal t ue changes wh le of value in differentiat ng the seases caused l y the viruses are not characters upon whi h a satisfa t ry clas i icati n of the viruses themselves

can be based Unfortunately only rarely is there sufficient information at hand to follow these criteria in a perfect or ideal manner Occasionally however it is possible to gather enough data and information to differentiate certain species and genera and to be reasonably certain as to their distinctness In these instances it appears logical to name and describe them taxonomically

According to the concepts presented in this paper the names of the insect viruses* (their descriptions have been published previously) may be listed in resume as follows

Order Virales Breed Murray, and Hitchens 1944
Suborder Zoophagineae Holmes 1948
Family Borrelinaceae Holmes 1948

Genus *Borrelina* Paillot 1926a
Species *Borrelina bombycis* Paillot 1926a (Type) (FIGURES 1 and 2)
Borrelina efficiens Holmes 1948 (FIGURES 3 and 4)
Borrelina reprimens Holmes 1948 (FIGURES 5 and 6)
Borrelina olethria Steinhaus 1949b (FIGURES 7 and 8)
Borrelina campeoles Steinhaus 1949b (FIGURES 9 and 10)
Borrelina peremptor Steinhaus 1949b (FIGURES 11 and 12)

Genus *Bergoldia* Steinhaus 1949a
Species *Bergoldia calypta* Steinhaus 1949a (Type) (FIGURES 13 and 14)
Bergoldia daboia Steinhaus 1949b (FIGURES 15 and 16)
Bergoldia lathetica Steinhaus 1949b (FIGURES 17 and 18)
Bergoldia thompsonia Steinhaus 1949b (FIGURES 19 and 20)
Bergoldia brassicae (Paillot 1926a) Steinhaus 1949a
Bergoldia clistorhabdion Wasser and Steinhaus 1951 (FIGURES 23 and 24)
Bergoldia nosodes Hughes and Thompson 1951 (FIGURES 21 and 22)

Genera et Species Incertae Sedis
Genus *Paillotella* Steinhaus 1949a
Species *Paillotella pieris* (Paillot 1926a) Steinhaus 1949a (Type)

Genus *Morator* Holmes 1948
Species *Morator aetatulae* Holmes 1948
Modator nudus Wasser 1942

Although for most of the nomenclatorial problems the International Bacteriological Code of Nomenclature may serve as a guide the problems of classification on the other hand are great and seemingly ominous It behooves us however to avoid the trap of despair hopelessness or defeatism and to approach the problem forthrightly energetically and in a cooperative and understanding manner remembering the admonition of Kipling 'Go to your work and be strong Stand to your work and be wise '

* Th w g a *Sm thia* proposed by Dr B g ld at th f ren wh h th s m g aph b d n t ... of D B rg ld ... t d t d t th t m ... th type spe ... ld ted by th d g t on ... of th ... Wh th g ... of type pecies t sh ld be dd d ... t th pr t list

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PLATES 1 to 4
(Figures 1 to 24)*

PLATE 1

Figure 1 Electron m crographs *Borrel na bombycis* Pa'llot the cause of j undice in the silkworm *Bombyx mori* (Linn.) Top ew show indi idual irus particles, and bundles of two particles each. *Lower view* (negative print) shows individual particles The virus particles a d bundles h ve been freed from th polyhedra by dissol tion of the latter in dil te sodium carbonate Approxim te magnification top view 27,500× lower iew 21 000× (From Bergold, 1948a b)

Figure 2 Polyhedra characteristic of silkworm j undi as seen with an rdinary light microscope. Magnification app o im tely 1,000× (Courtesy R. W Glaser)

Figure 3 Electron m crograph of *Borrel na ficiens* Holmes the cause of a polyhedrosis (*W pfelkrankheit*) of the non-moth caterpillar *Lymantria monacha* (Linn.) B dles predominate. Magnification approximately 16,800× (F om Berg ld 1948a.)

Figure 4. Preparation showing th polyhedra characteristic of the polyhedrosi of the nun-moth caterpilla Magnifi tion pproximat ly 460× (Courtesy R W Glaser)

Figure 5 Electron m crograph of *Borrelina r primens* H lmes, the cause of polyhedrosis ("wilt disease") of the gypsy moth caterpillar *Porthet sa d spar* (Linn.) Magnification approximately 37,500× (From Bergold, 1948b)

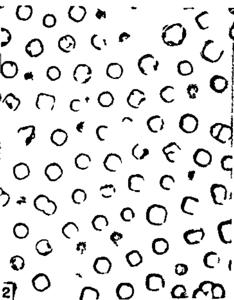
Figure 6 Polyhedra characteristic f the polyhedrosis of the gypsy-m th caterpillar Magnification approximately 1 000× (From Glas 1915)

Photographs 1 3 5 13 and 14 w re obtained through the courtesy of Dr B rgold. Photograph 2 4 and 6 were obtained through th courtesy of the lat Dr R. W Glaser Photographs 7 8 9 10 12 15 16 17 18, 20, 21 and 22 w re mad for the utho by Mr K. M Hughes and photograph 11 19 23 and 24 were prepared by Mrs H B Wasser

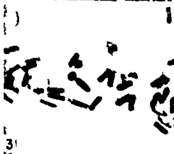




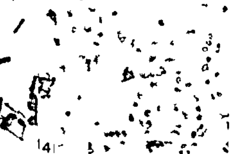
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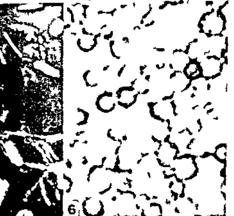
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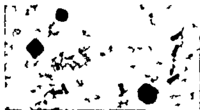
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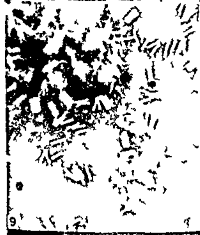
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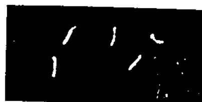

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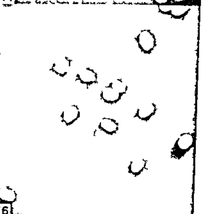

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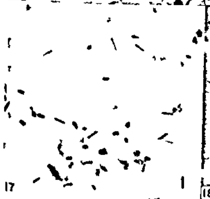


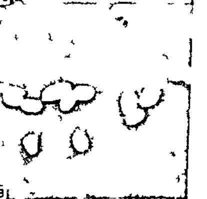

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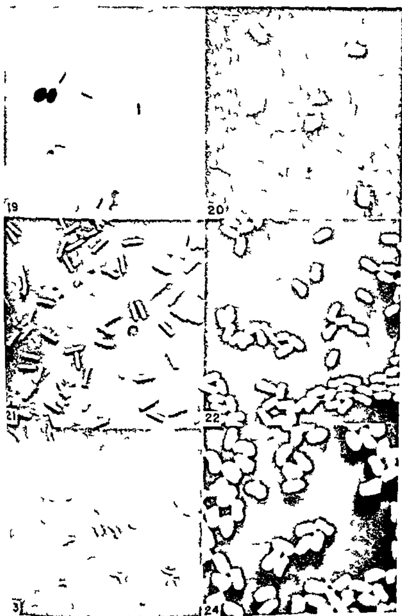


Plate 4

CRITICISM OF BINOMIAL NOMENCLATURE AS APPLIED TO PLANT VIRUSES

By F. C. Bawden

Rothamsted Experimental Station, Harpenden, Herts, England

The title of my paper was provided for me, so that I am unsure what significance to attach to some of the words and phrases. For example, more than one type of binomial nomenclature has been applied to plant viruses, and the word "criticism" *can also have more than one meaning. I think I am probably* safe in assuming that I am expected to criticize the Linnean type of nomenclature exemplified by *Marmor tabaci H.* rather than the older (for plant viruses) and more widely used vernacular type of binomial, exemplified by tobacco mosaic. For reasons that need not be gone into now, I also imagine *that the word "criticism" is meant to carry its most common current usage of* adverse criticism, even perhaps of carping, rather than its original and wider usage of evaluation. Nevertheless, I shall try to give it its wider meaning, although I expect many will consider I shall not try hard enough. Therefore I may as well begin, as I shall end, by saying that I think we should be very *unwise now to adopt Latin binomials for plant viruses.*

I reach this conclusion only reluctantly, because I am a biologist, and so Latin binomials have an attractively familiar air. Also, a system of naming that can reveal some attribute peculiar to a species as well as its relationship to like species sets a standard of usefulness not easily reached in other ways. *It is excelled only by the names used in inorganic chemistry, but it is vain for* the biologist to seek the precision of identity carried by such names as sodium chloride. That binomials now often fail to achieve their maximum usefulness because many names have no descriptive significance and many users are ignorant of Latin is a reflection on the users and no fault of the system. There is no need for me to *extol the virtues of the system, however. The facts speak* for themselves. Since it was first fully developed by Linnaeus in 1753, its use for plants and animals has become so firmly established that a change is almost unthinkable. It is perhaps worth noting, however, that Latin binomials, despite their evident merits, have not come into general use for things other *than organisms, and the early attempts to apply them to human* ailments and minerals soon failed.

The very success of Latin binomials with plants and animals may itself have militated against their more general use. Certainly it is one reason why I think they should be avoided with viruses, *at least for a time. The use of a generic name and specific epithet now* carries with it, from the classification of plants and animals, implications of relationships that are wholly unjustifiable from our present knowledge of viruses. This need not have happened, for species and genus could have been regarded simply as *levels of similarity* applying generally to the classification of ideas, languages, art, and inanimate *objects no less than to that of* organisms. Informally they sometimes are generally applied, but formally they have been applied only to organisms and

consequently have come to carry meanings implicit in the accepted method of classifying organisms For better or worse this method is the unique one in which phylogeny has come to be all important and for building genera from species families from genera and orders from families natural relationships and evolutionary origins take precedence After Dr Mayr's wholly admirable paper, there is fortunately, no need for me to emphasize the fact which seems to have been overlooked by many virus workers I need say only that without any knowledge of the phylogeny of viruses we have no right to adopt a system of nomenclature that suggests we do have such knowledge

Again this emphasis on evolution need not have happened Plants and animals could be classified in various other ways and to the farmer few classifications could be more futile than the biologists natural one Also Linnaeus lived before Darwin and made his classification without considering evolution This however is no reason for arguing that virus workers should now do similarly We are in the post Darwinian era but our relevant knowledge of viruses is in the pre Linnean stage of knowledge on plants and animals Although Linnaeus grouped arbitrarily so that many of his groupings have been abandoned others have not His groupings have survived largely because Linnaeus had advantages denied to the virus worker Using gross morphological characters as bases for grouping and separating inevitably meant that he often selected major evolutionary trends for such events as changing the numbers of carpels call for many combined gene changes events unlikely to happen frequently The persistence of the Linnean system is largely attributable to the fact that it could be adapted to showing the family tree relationships that have come to dominate biological classification

There seems to me only one logical argument for naming viruses on the Linnean system It is that the system is not only used but also works well with bacteria and that viruses are similar to bacteria I know too little about bacteriology to comment on the success of the nomenclature but I do know that almost every time I want to call a bacterium something that was acceptable the last time I used it the generic name has altered Also to keep workers informed not only are vast handbooks needed but also an international bulletin that is published quarterly This does not fill me with confidence that the system will work smoothly with viruses which have even fewer intrinsic characters on which to decide relationships

Nor is there anything substantial in the argument that viruses resemble bacteria It is true that the two can cause similar syndromes and raise similar epidemiological problems Also present evidence suggests that the particles of different viruses may almost cover the size range between the smallest organisms and normal proteins and one school of thought derives viruses from pathogenic bacteria by a progressive loss of form and function Ultimately this idea may be proved correct but it is now an unsubstantiated theory and others equally as plausible can be advanced There is indeed no reason to assume that all viruses have similar origins A time when widely conflicting theories can reasonably be held seems singularly inappropriate for adopting a uniform standardized nomenclature From current knowledge on

the nature of some plant viruses, it seems unlikely that they are degenerate organisms Their nearest analogues seem to be the macromolecular nucleoproteins of normal cells Are we to use pathogenicity as the criterion for deciding whether a nucleoprotein receives a Latin binomial? Is Lwoff's "prophage to be denied one to be awarded one after activation by ultraviolet irradiation? Or are we to give Latin binomials to all the macromolecular components of cells that are individually identifiable, so that these will bear names of the same kinds as their parent organisms? Surely we are better advised to leave viruses with their common names, which make no false pretensions at grouping into genera, and also avoid these awkward consequences

Supporters of Latin binomials often wax eloquent over the virtues or need of names to be in an "international language I shall not labor the point that fewer people now understand Latin than understand most current languages for I have much sympathy with the plea when it is made by botanists who are consistent enough to insist that descriptions of types are also recorded in Latin If virus workers are so misguided as to adopt Latin binomials however they will presumably follow the bacteriologists and while demanding Latin names, will accept descriptions in other languages as valid This reduces the argument to nonsense for an individual who cannot be expected to understand a name in English French Spanish or Chinese, is apparently expected to be able to reach his identification from details recorded at length in these languages

There is another reason why I think we would now be wrong to tie ourselves to a formal system of nomenclature We would immediately be bound by complicated rules of nomenclature at a time when virus research needs as much freedom as possible Those who think that Latin binomials are best should be encouraged to experiment with their method but we should keep the way open for testing other methods as well and avoid systems that will inevitably lead to many changes and occupy the time of many people to make them

The main purpose of a name is to act as a label for unequivocal reference If it can do more than this (as the binomial names undoubtedly can when fully applied to appropriate subjects) well and good, but let us remember the prime function of a name Article 4 of the International Rules of Botanical Nomenclature 1930–1935 reads ' The essential points in nomenclature are (1) to aim at fixity of names (2) to avoid or reject the use of forms and names which may cause error or ambiguity or throw science into confusion ' Point (1) would not seem to be fulfilled any better by Latin binomials than by common names Tobacco mosaic virus has remained in constant use for more than 50 years without causing any great confusion whereas three Latin binomials *Marmor tabaci*, *Musivum tabaci*, and *Baculus tabaci* have been proposed since 1939 Let us take warning from our bacteriological colleagues who have had longer experience of Latin binomials Crown gall bacterium is a name that has been a constant label and carried an unambiguous meaning for plant pathologists for nearly 50 years whereas the generic name in the Latin binomial has changed from *Bacterium* to *Pseudomonas*, to *Bacillus* to *Phytomonas* and lastly (at least if I am up to date) to *Agrobacterium* All these changes were no doubt justifiable but they do not exactly fulfill point (1) of Article 4

The desirability of permanent names is reiterated in Article 1? which reads "No one may change a name (or combination of names) without serious motives" and in the accompanying Recommendation III which reads "Changes in nomenclature should be made only after adequate taxonomic study" It may be significant that Article 1? and Recommendation III are omitted from the rules as printed in Bergey's *Manual of Determinative Bacteriology* the only standard manual that has yet used Latin binomial names for viruses It is particularly the omitted Recommendation that I think virus workers should consider carefully Can anyone seriously maintain that viruses have received enough taxonomic study to justify a complete change of nomenclature? It is indeed less emphasis on nomenclature and more on taxonomy that is needed If anyone doubts this one look at Holmes's genera of plant viruses should be enough to convince him Not only do viruses about whose intrinsic properties nothing is known get the same generic name (at least this does not conflict with knowledge) but so do many viruses that are known to differ widely both in their intrinsic properties such as morphology chemical constitution and physicochemical behavior and in properties that may or may not be intrinsic such as method of transmission host range and pathogenicity

Before we can classify viruses let alone give them names that suggest they have been grouped on natural relationships we need to decide what criteria are to be used This is what people should be busy trying to do rather than inventing new names however ingenious and attractive these may be A Latin translation of a common name may sound more learned and exact than the original but it is still a common name On what appears to be the main principle by which binomial names have so far been applied to viruses cow parsley and the cow oak would probably find themselves in the same genus perhaps sharing the generic name *Bos* No doubt botanists could make many comments about deriving genera for such reasons but they can be summarized adequately with this example by saying that the name seems to lack a final "h.

Although I sincerely hoped that the schemes of nomenclature and classification that were put forward by Smith and Holmes would not be adopted by virus workers nevertheless I welcomed their appearance because I assumed they would encourage or irritate workers to consider taxonomy more carefully than has been usual hitherto This assumption has proved entirely unfounded Without doing a hand's turn more to identify the virus with which they are concerned workers who follow the binomial nomenclature merely give it a fancier name I will quote one typical example from a recent number of *Phytopathology* which contained a paper dealing with a disease of fuller's teasel (*Dipsacus fullonum L.*) The author described symptoms on a few hosts, his failure to transmit the cause by sap inoculation and success with two species of aphids From this he then stated that "it is believed that the teasel mosaic disease is caused by a hitherto undescribed plant virus and the binomial *Marmor dipsacum* is proposed Imagine a mycologist behaving similarly finding a diseased teasel and getting evidence that the cause was wind borne and then stating that "it was believed to be caused by a hitherto

undescribed fungus for which the name *Puccinia leashi* is proposed' And now, if your imaginations are sufficiently vivid, imagine what his mycological colleagues would say I can see no reason why virus workers should accept standards that would not be tolerated by workers in other disciplines It is our duty to treat such pretentiousness with the scorn it deserves In the old days of virus research, we might even have demanded data about filterability or absence of a visible pathogen, before admitting transmissibility as evidence of a virus disease and then the cause would certainly have received some such name as teasel mosaic virus' or "teasel virus 1' These names express the position exactly a virus that infects teasel and may be distinct from any previously described, but may equally well be a strain of one already named In other words names that fit the knowledge about the things named

I trust that my emphasis on the desirability of common names for plant viruses will not lead anyone to conclude that I think all is well with virus nomenclature and that nothing needs doing This is far from the truth and more often than most, I have bemoaned the chaotic state we have reached It is, indeed distressingly obvious that virus nomenclature does not fulfill point (2) of Article 4 of the rules for it contains many examples of errors and ambiguities and of "science being thrown into confusion The chaos however has not arisen primarily because of the type of nomenclature but because of the way it has been used or rather misused Long after it was well established that some viruses have wide host ranges and occur in strains that cause different syndromes, workers continued to behave as though a previously undescribed symptom was adequate for claiming and naming a new virus Most of the errors could have been avoided had a wider range of more critical tests been made before a name was applied There is no excuse for the scores of synonyms that have been applied in the last 20 years to such viruses as tobacco mosaic, potato X, and cucumber mosaic when unequivocal methods of identification existed but remained unused A change in the type of nomenclature is not going to reduce the chaos The only way order will be achieved is by workers approaching their identifications more critically and cautiously than they have hitherto A change to Latin names now can only make things worse by creating another lot of new and unnecessary synonyms when our prime need is to reduce synonymity My plea is not that we should do nothing but that we should do what is most likely to produce a useful result When lost in a fog there is nothing to be gained by weighing anchor and sailing full steam ahead in the wrong direction The journey is likely to end sooner by staying put until the fog clears and some accurate bearings can be obtained For virus nomenclature the bearings of prime importance are tests to ascertain whether clinically distinguishable viruses are related strains or not

Fortunately, there is now good reason to think that grouping variants or strains around type viruses will not be too difficult As James Johnson pointed out more than 20 years ago tests of stability *in vitro* are often more valuable than symptoms as bases for identification but to such tests we can now add the more specific one of serological relationship and the more widely applicable one of mutual antagonism No doubt as these tests are used more examples

will be found where they are not fully reliable but there is already enough evidence that they apply sufficiently widely to get rid of most of the synonyms that now confuse the literature The validity and usefulness of these tests was recognized by Holmes in deciding his species even though putting together two clinically different strains that are serologically related or mutually antagonistic sometimes meant conflicting with the bases on which the genus or family was erected. Indeed he performed a very useful service by grouping strains under one specific name as far as was possible on published evidence Where his scheme fails however is in adopting the binomial nomenclature for this demands groupings at the generic level and there is not enough knowledge to do this This fact is only too evident from the heterogeneity of Holmes s genera We can group at the species level but we cannot yet go beyond this with any degree of confidence Lacking any knowledge of the intrinsic properties of most viruses whether we like it or not we must be splitters and not lumpers if we are to avoid false names Holmes was a lumper *par excellence* to such an extent that a generic name like *Marmor* carries no meaning at all The problem of lumping is best posed by asking what virus not serologically related to tobacco mosaic virus is most nearly related to it? It is unanswerable but I could make out a better case for potato virus X which Holmes places in a separate family than for most that he places in the same genus

It seems to me that a binomial nomenclature for viruses can be worked only if most of the genera are monospecific and what is to be gained by that? Or we might work the second and third names of a trinomial nomenclature using a standard form, say *Phytovirus* for the first Then tobacco mosaic and tomato aucuba mosaic viruses could be respectively *Phytovirus paracrystalis* var *vulgare* and *Phytovirus paracrystalis* var *aucuba* and cucumber virus 3 *Phytovirus paracrystalis* var *cucumis* Suitable specific and varietal names could readily be given to other viruses and their more studied strains but the only benefits apparent to me are that such names are less likely to get translated and look more impressive than current ones plus the possibility that the identity of the namer will be handed down to posterity Against these minor benefits can be set the implicit separation of plant viruses from those affecting animals a separation that has never had any sound taxonomic basis and looks even less tenable now that some viruses have been shown to multiply in both plants and insects

Names of this type share with common names the virtue that they suggest no false relationships and leave open opportunities for experimenting with various forms of classification The binomial nomenclature almost necessarily calls for a detailed knowledge of the intrinsic properties of viruses because only from these can a classification be built that reflects natural relationships and evolutionary trends We are beginning to learn something about the intrinsic properties of a few plant viruses but it will be long before we know anything about the majority and we know almost nothing about origins We do however need to encourage the arrangement into groups of viruses with like behavior for order is more easily comprehended than disorder and arrangements aid identification and stimulate research Reliable keys to identification

are probably the most urgent need Many years ago Johnson and Hoggan showed that such keys do not require a binomial nomenclature and can lead to groupings as homogeneous as any families or genera since propounded Free from the shackles imposed by Linnaean names, we need not be prevented from making groups by ignorance of intrinsic properties for we can, without misleading other biologists, select any convenient feature Symptoms host ranges, vectors, methods of prevention or cure, all such types of behavior could provide bases for useful grouping

Without generic names, the groupings arranged for identification keys can differ widely from those arranged for other purposes There can be several kinds of classification based on different criteria, to suit the purposes of people interested in viruses for different reasons For each, it is necessary only to decide what purpose the classification is to serve, and then to group accordingly Many people are concerned with viruses in many different ways and few will be helped in any practical manner by a phylogenetic classification No other type demands a nomenclature of Latin binomials which should be restricted to that type Such a classification of viruses is likely to be long delayed but this is no reason to despair of doing anything now On the contrary it should be a *stimulus for seeking different types of classifications By all means* let us resolve to put our house in order, but we must not pretend we can do some thing that we cannot Nor is there any need for the pretense when we can probably *do something much more useful*

PSITTACOSIS GROUP

By K F Meyer

George Williams Hooper Foundation Un versity of California San Francisco Calif

The pandemic of human psittacosis in the early thirties initiated the productive experimental investigations concerning the cytoparasite responsible for this infection Simultaneously in Britain America and Germany small coccoid bodies were seen in suitable stained smears of infective parrot or human tissues Since these bodies could not be cultivated and do pass through coarse bacteriological filters they were classed at first as agents morphologically related to *Bacterium tularense* or to the Rickettsiae The pleomorphism expressed in bipolar or coccal forms of varying sizes prompted Levinthal (1930) to propose the name *Microbacterium multiforme psittacosis* The word multiforme was not entirely appropriate for the pleomorphism is in no way more pronounced than that ordinarily seen in smears prepared from other small bacteria Since arthropods do not act as vectors the name *Rickettsia psittaci* offered by Lillie (1930) was likewise not acceptable Thus the problem of nomenclature and classification arose nearly 20 years ago when the virus was first described and it has plagued the microbiologist ever since

In order to avoid any premature decision the workers in this field called the elementary bodies constantly associated with psittacosis infections Levinthal Coles Lillie (L C L) bodies' This tentative nomenclature had the great advantage that it avoided the word psittacosis originally given in 1892 by Morange to the human disease following contact with parrots or parakeets How inadequately the name psittacosis is understood may be seen in the proposal to apply the provisional term pseudopsittacosis to those atypical pneumonias caused by 'psittacosislike agents of undetermined origin

There appeared to be no urgency for tampering with the name L C L bodies until Findlay MacKenzie and MacCallum (1938) postulated the existence of a definite life cycle analogous to that of the psittacosis parasite for the granulocorpuscles demonstrated by Miyagawa and his associates (1935) in lymphogranuloma venereum infections Subsequently in studies on the developmental cycle and other properties of the agent of lymphogranuloma venereum Rake and Jones (1942 1944) reviewed the work of Thygeson (1934) on trachoma and inclusion blennorrhea While these agents were subjected to systematic comparative studies others morphologically similar to the agents found in psittacosis or ornithosis were isolated from the pulmonary tissues of apparently normal mice (Gönnert 1941 1942 Nigg 1942 Karr 1943 Nigg and Eaton 1944) from cats suffering from a distemperlike disease (Baker (1942) and from the pneumonic lesions in certain cases of atypical pneumonia (Eaton *et al* 1941)

It is of interest to note that these isolations were doubtless favored by the recognition that in mice the intranasal route of inoculation is far more sensitive for detecting the L C L agents in secretions and in tissues than the customary intraperitoneal or intracerebral route originally employed by the students of

these infections The ability to produce pneumonia in experimental infections and the fact that the agents frequently were associated with pneumonitis in the spontaneous disease impressed the investigators to such an extent that the nomenclature was promptly enriched by the term "pneumonitis group" of viral agents Indistinguishable morphologically, but slightly different anti genically, agents from the type strains of the classical psittacosis parasite used for comparison were invariably described as 'pneumonitis viruses when epidemiologically the source of the human infection or illness could not be determined At that time it was not appreciated that *pneumonitis is the* localizing sign of a clinical infection with a psittacosis ornithosis or related agent in man and mammals *but not in birds* *Thousands of autopsies on* parrots parakeets pigeons ducks canaries and other birds spontaneously diseased or experimentally infected by the nasal route have failed to disclose primary uncomplicated pneumonic lesions

Then another property of the large viruses was singled out and chosen in a proposed classification Impressed by the ability of certain strains of L C L agents to produce meningitis and paralysis in mice and pigeons some authors were prompted to select the tropism of the parasites and to refer to them as meningopneumonitis or meningo-pneumotropic viruses (de Gara and Furth 1948) Although the brain of the paralyzed Colombian opossums contained no infective agent the cause was described as a meningopneumonitis virus because it produced meningitis or pneumonitis in mice, depending on the route of infection (Roca García 1949) There is doubtless merit in selecting tissue tropism for identifying and separating the members of a group of infective agents All the parasites morphologically similar to the L C L bodies produce a pneumonitis in the laboratory mouse when injected by the intranasal route This is also a property of the agent isolated from the intestines and feces of apparently healthy calves (York and Baker 1951), and the parasite which by its localization in the *epithelial cells of fetal cotyledons causes abortion* in ewes (Stamp *et al* 1950 McEwen *et al* 1951 Barwell and Bishop 1951) However spontaneous pneumonitis in mice and occasionally in other mammals is a group or family *characteristic that manifests itself only* when the portal of entry is the upper respiratory tract Furthermore the infection through the respiratory tract does not remain confined to the respiratory tract systemic transfer to the *spleen and liver is the rule* This *infection* cannot be relied upon to separate the members of the group of Castaneda positive L C L bodies The agents of murine pneumonitis of enzootic abortion of ewes the so-called S F *virus isolated from* cases of human pneumonitis and the feline pneumonitis parasite when inoculated by the intracranial route rarely if ever produce apparent infection even though they may persist in *the brain for several days* Exceptions have already been encountered At least two mouse pneumonitis strains that are virulent for mice by the intracranial route have been isolated (de Burgh *et al* 1945 Eddie and Meyer 1948) *Host* and tissue *tropism may* be useful for quick identification but they cannot be relied upon to separate the members of the group of Castaneda positive L C L body agents

In summary the available facts permit definite conclusions During the past 20 years elaborate and painstaking studies have pointed out the existence of infective agents which share striking similarities in morphologic and tinctorial characteristics Although they are responsible for frank and latent infections in the animal kingdom including man they are ...ely related and have in common certain antigenic components evidenced in the complement fixation cross-immunity and neutralization tests especially in their so-called toxic properties There is no difficulty in deciding that they should be classed into one group characterized by the following properties They are relatively large parasites and are readily stained with simple basophilic dyes therefore frequently called Castaneda positive They form large basophilic inclusions in endothelial and epithelial cells They grow well in the yolk sac of embryonated eggs They infect a wide host range and produce pneumonitis in the laboratory mouse when introduced by the intranasal route Certain members of the group produce what seems to be an endotoxin These appear to be antigenically related although distinctions are discernible They are susceptible to the action of the new chemotherapeutic drugs

The problem is to find an appropriate name for this group Endless solutions have been advanced Designations from cytomicrobe (de Cara and Furth 1948) to trachoma pneumonitis-psittacosis-ornithosis-lymphogranuloma venereum group have been proposed Dependent entirely on the specialty or special interest of the designator the sequence of the genus names is shifted The ophthalmologist describes the trachoma psittacosis lymphogranuloma venereum group and the dermatologist puts the lymphogranuloma before the psittacosis This juggling of a long worded designation of a group of infective agents has even led to abbreviations such as psittacosoid viruses causing human pneumonitis The word means parrot like This usage is obviously an abomination The workers who know most about this group have always felt that it is semantically reprehensible to drag in the parrot every time an agent that forms basophilic elementary bodies of a certain size shape and staining properties is isolated To end all of this unsatisfactory and jarring nomenclature the terminology of Moshkovsky (1945) was adopted in 1948 and the family name Chlamydozoaceae and the genus name *Miyagawanella* were made part of Bergey's System It may be helpful to future development of the taxonomy of viruses to consider this provocative terminology and classification from several particularly the historical points of view To clear the way and to gain strength for this presentation it is only proper to quote the following lines from the excellent book by Bedson Downie MacCallum and Stuart Harris (1950) Virus and Rickettsial Diseases

"Recently Moshkovsky (1945) has suggested that the Castaneda positive viruses should be given the generic name of *Miyagawanella* in the family Chlamydozoaceae and this suggestion has unfortunately been adopted by those responsible for Bergey's Manual of Determinative Bacteriology It is not proposed here to adopt Moshkovsky's classification

According to Andrewes (1951) the Fifth International Microbiological Congress at Rio de Janeiro in August 1950 named five well-defined groups of

viruses The "psittacosis lymphogranuloma group" (Chlamydozoaceae) was included as it had received attention by Moshkovsky (1945) and by Rake in the 1948 Bergey's Manual This decision to shorten the designation of the group is a step in the right direction but the retention of the family name 'Chlamydozoaceae provokes discussion

Representatives of the large viruses related to the Castaneda positive cytoparasites have been the subject of painstaking morphological studies—for psittacosis by Bedson (1932, 1933) and Bedson and Bland (1932, 1934) for lymphogranuloma venereum by Rake and Jones (1942, 1944), and for the feline virus by Weiss (1949, 1950) and Moulder and Weiss (1951a–c) A life cycle involving large and small forms, an apparent envelope or limiting membrane, relative ease of cultivation, and their susceptibility to chemotherapy place them for the time being by themselves The newer electron microscope pictures show their morphologic resemblance to the rickettsiae, although the elementary bodes of the psittacosis agents are coccoid and spherical, never rod shaped (van Rooyen and Scott 1949 Kurotchkin *et al* 1947 Heinmets and Golub, 1948), but they differ from rickettsiae in that none of them have an arthropod host Furthermore, old (Yanamura and Meyer, 1941) and new (Burney and Golub, 1948 Moulder and Weiss, 1951b) investigations indicate that the psittacosis and the feline pneumonitis viruses are apparently dependent on tissue survival and a high level of host cell metabolism for their multiplication Recent work fully confirms the early observations of Zinsser and Schoenbach (1937) that rickettsiae grow best in cells in which metabolic activity has almost ceased In chick embryos the rate of multiplication of typhus rickettsiae is within reasonable limits inversely proportional to the rate of host-cell respiration (Greiff *et al*, 1944, Greiff and Pinkerton 1945, 1948) Moreover the published record indicates that rickettsiae possess intrinsic enzymes of some complexity suggesting the partial metabolic independence of these organisms (Bovarnick and Snyder, 1949, Wisseman *et al* 1951), while preliminary search for similar enzymes in the agent of feline pneumonitis have failed (Moulder and Weiss 1951a) If these differences which include the nonsusceptibility of the psittacosis virus to *p*-aminobenzoic acid are confirmed the agent of the 'psittacosis-lymphogranuloma venereum group may have to be placed in the virus fold The differences between the psittacosis lymphogranuloma venereum group and the rickettsiae are rapidly being extended at the basic level of metabolic requirements of the parasite Though these differences are marked the present class grouping presented in Bergey s Manual is acceptable as a working relationship, but may require change as greater independence of rickettsiae may be demonstrated

The next step in coming a descriptive and correct terminology for the psittacosis lymphogranuloma venereum group requires consideration of the family name Chlamydozoaceae

In 1907 to 1912 van Prowazek united, under the name of *Chlamydozoa*, a group of minute intracytoplasmic bodies unlike any previously known disease agents which he and Halberstaedter had seen in the conjunctival epithelial cells from trachoma Those who lived through this period and saw the original

preparations vividly recall the discuss ons carried on then over the term The younger protozoologists fully realized almost immediately that the designation —mantle (chlamys χλάμυς) animals (zoa ζῷα)—was a mistake In the first place the basophilic bodies are not protozoa and in the se ond the so-called mantle or capsule is formed not by the microbe or agent but by the cell

Alert cytologists soon recognized that the inclusion body consists of three parts (1) the so-called ' plastin like substance a derivative of the cell cytoplasm, (2) the parasites and (3) the structureless matrix in which the elementary bodies are embedded Moreover in larger inclusions the plastin is rarely seen and the sub tance which is termed matrix is not related to the elementary bodies. It is merely the medium in which the elementary bodies multiply just as it is in molluscum contagiosum The plastin substance apparently represents the defensive mechanism of the host ce l and the nutritive medium used by the parasite (Halberstaedter 1917)

In Moshkovsky's chapter on the development of Chlamydozoa and in the discussion of the relationship of elementary and initial bodies he says

The further study of this problem soon led to a considerable simplification of the agents of the Chlamydozoa group and about their relation to the affected cell Already in the lifetime of Prowazek it was elucidated that the products of the reaction of the cell the obligatory presence of which around the parasite was thought as a characteristic peculiarity of this group of microbes can also be absent Lipschutz 1919, pointed out the paradoxicality of that circumstance that just in trachoma, which served to Prowazek as a prototype of Chlamydozoic infections no 'cloak (chlamys) of protoplasmic origin is formed and that on the basis of Lindner s work the inclusions of Prowazek Halberstaedter should be considered as naked aggregates of bodies of the agents which was acknowledged also by Prowazek *By that* by the way *fails off also the basis for the name Chlamydozoa* [Italics mine]

Notwithstanding this definite conclusion the name Chlamydozoa was retained by him and there is every reason to fear that it will now be permanently adopted in naming and classifying a group of cytoparasites that are far better understood at this time and have been far more carefully studied than those of trachoma or inclusion conjunctivitis

Resemblance in morphological and tinctorial properties of the elementary bodies has been used to place viral agents of ophthalmologic interest with the psittacosis-lymphogranuloma venereum group It may be heresy therefore to question the soundness of the pillars on which this relationship is based The fact however that the 'plaques' give a positive glycogen reaction (Rice 1936) is rarely stressed The early views based on limited observations that most members of the group respond to chemotherapy with the sulfonamide drugs have failed to withstand the test of time The true psittacosis feline pneumonitis meningopneumonitis opossum A and calf enteritis agent are not sensitive to the drugs in mice The low complement fixation titers reported by Rake Shaffer and Thygeson (1942) with sera of individuals infected with trachoma or inclusion blennorrhea in the presence of a powerful lymphogranuloma venereum antigen (Lygranum) are not necessarily specific. On re-ex

amination of this problem using a psittacosis antigen, the sera from ten trachoma patients from Arkansas, tested at the Hooper Foundation, did not give significant complement fixation reactions. The sera of only one patient fixed complement in a dilution of 1:8. This patient had stage III trachoma. Three of five sera from patients with acute trachoma infections observed in Gallup, New Mexico, reacted in a dilution of 1:16 and one reacted in a dilution of 1:2. The recent literature, in particular from Denmark where lymphogranuloma venereum is a rare disease, places on record the important fact that positive complement fixation reactions with Lygranum antigen is often found in the absence of infection (Reyn, 1951). Similar observations have been made in Holland by Dekking (1949). The specificity of these reactions has not as yet been evaluated and, consequently, the low titers of fixation with sera from trachoma patients apparently do not necessarily prove antigenic relationships to the psittacosis-lymphogranuloma venereum group. If such a relationship really exists, it might be expected that in cases of trachoma the Frei test would be positive, but reported and unreported observations indicate that the reaction is negative (Rake *et al.*, 1942).

Trachoma differs from the psittacosis lymphogranuloma viruses in host range, ease of cultivation, antigenic relationship, and chemical reaction products induced in host cells. These differences are sufficient to recommend re-examination of (1) any designation extended from trachoma to psittacosis and lymphogranuloma venereum viruses, and (2) the unitary (generic) relationship of the inclusion conjunctivitides with the psittacosis and lymphogranuloma viruses.

The family name "Chlamydozoaceae" was mistakenly assigned to trachoma. Since the agent of this infection is not a "mantle animal," the poorly chosen name should not be perpetuated by applying it to the complex group of the large cytomicrobes that have nothing in common with protozoa. Regardless, therefore, of any other objections against this family designation, it is clearly unjustified, at this time, to bring the psittacosis lymphogranuloma venereum viruses into this family under a name given the agent of trachoma because its relationship to the large better-defined group is questionable. An appropriate designation should be selected on the basis of the pooled opinion of experienced taxonomists of an international body as soon as distinctive family characteristics become more clear cut.

All the veiled and open displeasure which can be voiced against the family name "Chlamydozoaceae" can be voiced with equal force against the genus name "*Miyagawanella*." The explanatory sentence in Bergey's Manual justifying this term makes it clear that it was adopted because the French parasitologist, Brumpt, in a footnote to an article on "Rickettsia intracellulaire stomacale (*Rickettsia culicis* n. sp.) de *Culex fatigans* (1938)," enthusiastically acknowledged the courtesy extended to him by Professor Miyagawa by showing him the intracytoplasmic inclusions he discovered in diverse specimens of lymphogranuloma venereum in the following words:

"Je crée ce dernier genre (Miyagawanella) pour l'espèce M. Lymphogranulomatosis n. sp., agent du bubon climatique, dont le Professeur Miyagawa m'a montré et offert des préparations démonstratives à Tokyo, en novembre 1935."

The statement in Bergey's Manual (p. 1115) that the genus was named in

ho or of Professor Miyagawa (the Japanese bacteriologist who in 1935 first grew the type species in the chick embryo) though perfectly true does not reflect the original reasons expressed by Brumpt for so identifying it. Actually there is some doubt about who first saw the elementary bodies responsible for lymphogranuloma venereum. Gay Prieto (1927) described small corpuscles 1 μ or less in diameter which differed from the Gamma Favre bodies. By showing that some of the cytoplasmic corpuscles are Castaneda positive Findlay (1933) proved more conclusively than Gay Prieto the relationship of these bodies to the virus of climatic bubo. Full credit must be given to Miyagawa and his associates (1935) for demonstrating in filtration experiments that the presence of these bodies paralleled infectivity but this fact had been established for the psittacosis virus three years before in 1932. It was left to Findlay and his associates (1939) however to suggest a possible cycle of development similar to that described by Bedson and Bland (1932) for the psittacosis virus. In fact the systematic studies of these workers accrued several years before Miyagawa described the elementary bodies of lymphogranuloma. Historical objectivity leaves little doubt that the elementary bodies associated with parrot fever were observed and properly interpreted before those of the venereal disease. The more common colloquial nomenclature psittacosis-lymphogranuloma group, credits this sequence. Accepting this premise it is further important to note that Burnet (1934) first propagated the Australian psittacosis virus on the chorio allantoic membrane. These cultivation studies were further developed by Fortner and Pfaffenberg (1934) and by Burnet and Rountree (1935).

All these facts cast doubt on the advisability of selecting the cytoparasite of lymphogranuloma as the type species which includes viruses distributed widely throughout the animal kingdom. Although no criterion for classifying the members of the group has yet been unanimously accepted as valid it must be realized before it is that the elementary bodies responsible for psittacosis in birds and man were the first well-defined member of the group. It is therefore only wise to resist applying the laws of priority to a name created in an epoch when knowledge concerning the members of the group was incomplete. A genus that has members infective for birds ruminants rodents marsupials and man should not be burdened by the name of an investigator who recognized but a few of the properties essential for the classification of any group. The noncommittal use of a proper name is considered preferable (in view of the wide host and tissue range of the members of the group) to a name implying a relationship to a bird genus or to a human disease. By custom the early investigator of the basophilic elementary bodies as infective agents deserves recognition. His name is traditionally reflected in the terminology.

Even here one is confronted by dilemmas.

Discovery and description of the presence of minute gram negative cocco-bacillary bodies in infected parrots by Levinthal (April 5 1930) in human and bird specimens by Coles (April 12 1930), and in parrot and human tissues by Lillie (April 11 1930) were obviously simultaneous. Their etiologic relationship to psittacosis was not definitely established by these descriptions. As already mentioned through the thirties it was customary to refer to the

causative agents of psittacosis as "L C L bodies" After the arrival of news about the cytoparasite of lymphogranuloma venereum and its relation to the psittacosis virus, the former name was silently dropped

In search of a possible solution for the problem of terminology and in an effort to learn the true historical development of knowledge of the basic facts about the psittacosis-lymphogranuloma venereum group of Castaneda positive elementary bodies, the literature on these viruses has again been read critically The following has been learned

As early as 1930, in a report submitted by Sir George Newman to the Minister of Health, the Right Honorable Arthur Greenwood in October, 1930, in "Part II—Aetiology—Experimental Observation on Psittacosis," Bedson and Western make this significant statement (p 88)

"Perhaps the most interesting observation bearing on the nature of this virus is the presence of minute bodies, but quite definite micro-organismal like bodies in virulent material both filtered and unfiltered We have ourselves studied them and, from an examination of a considerable amount of material we have formed the opinion that there is a definite correlation between their number and the virulence of material"

Then by January, 1932, Bedson had proved (1) that the "elementary bodies of psittacosis" are Castaneda positive, (2) that the virus is filtrable and may be purified by fractional centrifugation as illustrated by microphotographs, (3) that the washed virus particles are specifically agglutinated by an anti psittacosis serum, and (4) that they fix complement in the presence of such a serum In rapid succession Bedson and Bland (1932) and Bedson (1933) saw the relationship between the diverse forms of the psittacosis agent and the different stages of infection and were the first to realize that this virus passes through a developmental cycle Shortly thereafter, Bedson (1935 1937) conducted the basic studies that established the value of the complement fixation test in the diagnosis of psittacosis Finally, Bedson (1936, 1937) proved that the psittacosis virus contains two antigens one labile and readily destroyed by, the other resistant to, boiling

It was Bedson who furnished the first adequate proof that the elementary bodies cause psittacosis It would therefore seem appropriate that Professor S P Bedson's name be associated with this group The name *Bedsonia* is here proposed as a generic title for all the agents now grouped under the name psittacosis viruses of avian human and mammalian origin The ultimate classification and terminology for the cytoparasite responsible for lymphogranuloma venereum should be deferred until the biologic properties of a greater number of strains from a greater number of sources have been studied Certain differences in staining reaction require further elucidation The elementary bodies of lymphogranuloma venereum though Castaneda positive give no reaction by the Feulgen method (Robinow and Bland, 1938) while the psittacosis virus is positive by both methods Reported differences noted with two strains (one European and another American), with respect to immunogenic properties, as appraised by cross-immunity tests and difficulties of propagation in the yolk sac may or may not be significant (Lépine and Sautter 1949) Whether a generic name should be used to cover the psittacosis *and* the lympho

granuloma viruses is definitely another matter for discussion and a final decision might well await the result of further work

There is no need to dwell further on the limitations of the present listing of species of large viruses presented in Bergey's classification An analysis of the results brought to light by the various techniques employed in the identification and classification of members of the psittacosis group furnishes ample warning against the hasty creation of new species Neither host or tissue tropism nor sensitivity to drugs is a dependable property for classification in this group Reliance may ultimately be placed on antigenic differences especially the so-called toxic properties For example there are indications that sero types revealed by toxin antitoxin neutralization tests may break up the species ornithosis

One also wonders whether it is accurate to single out the Louisiana Illinois or San Francisco viruses as separate distinct species If this is done some place has to be found for the virus isolated by Yeatman and McEwin (1948) and for the one isolated by de Gara and Furth (1948) The first named investigators observed in Adelaide Australia four cases of severe acute atypical pneumonia A psittacosislike agent was isolated but because of the absence of any evidence of an avian source of virus they hesitated to name the agent even though pathogenicity tests placed the virus with the highly virulent psittacosis strains Yeatman and McEwin emphasized that it was impossible to eliminate brief contacts with birds in a country where psittacosis is a rather common infection among parrots According to de Gara and Furth the patient whose autopsy furnished a psittacosis virus knew that he had not been in contact with birds Wisely, no attempt was made to christen this virus which according to the published record resembles the strains isolated from pigeons Similar observations are continuously made by those who systematically inoculate mice with tissues from patients with pneumonitis As a rule similarity with avian strains is more marked than differences recorded for strains isolated from the same species of birds Classification of such strains should be deferred until sound criteria for a taxonomic approach have been worked out

At the earliest possible moment consideration must be given however to the poorly chosen difficult-to-pronounce terminology of classification adopted from the unfortunate treatise of Moshkovsky

Summary and Recommendations

The large intracellular parasites—(1) responsible for diverse spontaneous clinical and latent infections in birds, mammals and man (2) readily stained by basophilic dyes, (3) antigenically related as evidenced in complement fixation cross-immunity and toxin neutralization tests (4) capable of producing pneumonitis in the laboratory mouse when introduced by the intranasal route (5) growing well in the yolk sac of the embryonated egg and (6) susceptible to the action of certain chemotherapeutic agents—form a distinct biologic group Since the basic characteristics of this group were recognized first in the agent responsible for psittacosis and not until several years later in that of lymphogranuloma venereum the group should be designated the

psittacosis lymphogranuloma venereum group The investigator Bedson responsible for the elucidation of the morphologic, physiologic, and immunologic properties of this group should be recognized, and the nomenclature proposed by Moshkovsky and, *unfortunately*, adopted in the Bergey System should be discarded The criteria for placing in this group the known agents and any others that may be discovered should be defined by an international group of workers familiar with these parasites The creation of a family genera and species by expert taxonomists should be deferred until further knowledge justifies this step

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THE LYMPHOGRANULOMA PSITTACOSIS GROUP

By Geoffrey Rake

The Squibb Institute for Medical Research New Brunswick N J

As will become clear it is my opinion that if viruses and rickettsiae are to be separated then the group I shall discuss belongs with the latter rather than with the former I have given long consideration to the classification and nomenclature of this group of agents and of certain others related to them Indeed such consideration may be said to date from the time when my colleagues Shaffer and McKee, and I[1] first obtained abundant amounts of the agent of lymphogranuloma by egg culture and came to realize its relationship to the agent of psittacosis That is now 12 years ago As this relationship became clearer and as other members of the group became isolated and characterized it became obvious to many of us working with these agents that there would be a considerable convenience if a suitable nomenclature could be found and that such convenience might outweigh the relative imperfection of knowledge on which classification and therefore such nomenclature could be based

Accordingly a group of investigators in this field chiefly American began a study of the matter Doctor K F Meyer whose paper is also included in this monograph (p 545) was one of the group It soon became clear that to obtain unanimity of opinion in such a group was fraught with great difficulty Indeed after long striving sufficient unanimity had not been obtained to allow the publication even of a tentative and skeleton system

Meanwhile two events occurred The first was the publication by Moshkovsky in 1945 of a system of classification and nomenclature for this group of agents and of others which to him appeared related[1] In common with the general thinking in Europe he classified these agents with the rickettsiae The second event was the decision by the authors of Bergey s Manual to include in their next and shortly forthcoming edition both the rickettsiae and the viruses The present author was invited to undertake the classification and nomenclature of the lymphogranuloma psittacosis group for the new Manual[2] It was indicated to him that a tentative opinion of the editors of the Manual put these agents with the rickettsiae and not with the viruses This opinion coincided and still coincides with that of the writer but frankly it is one of the points on which the abortive American committee was unable to agree Three opinions were held within that committee (1) that the agents were viruses (2) that they were rickettsiae and (3) that the matter was of no significance because there was no good reason to distinguish between viruses and rickettsiae at least at any high level within a system of classification

Since as I have indicated above I believed and still believe that the agents are closer to the rickettsiae than to viruses let me occupy the first part of this presentation in justifying such an opinion In morphology at least the morphology of the usually observed form there is no similarity between the rod shaped rickettsiae and the coccoid Chlamydozoaceae (to introduce now a name I shall discuss with you below) However both agents go through a series of

developmental stages within the host cells and many of the early stage development are morphologically closely similar for the two groups[4] tron micrographs show marked resemblances between rickettsiae and *wanellae*[6, 7] particularly in the appearance in both groups of a centr mass which shrinks away from a well-defined limiting membrane connection also, attention may be drawn to work by the present autl various colleagues on the mode of multiplication of the agent of lymp loma on the one hand, and molluscum contagiosum on the other, within cell The earliest stages of multiplication of the agent of lymphogra venereum within the yolk cells of the developing chick embryo hav studied and binary fission (with first pairs, then tetrads and later, c has been clearly demonstrated[4] While the exact details of multip of the virus of molluscum contagiosum are not yet available it is clea in common with other viruses the fundamental details are different th tose nucleic and desoxypentose nucleic acids are altered throughout th plasm and morphologically, one can distinguish the appearance of previral units[8]

In tissue tropism the *Miyagawanellae* resemble the Rickettsiaceae i predilection for the yolk cells of the chick embryo over all other tissues host No viruses share this predilection Tinctorial reactions of the Cl dozoaceae are almost identical with those of the Rickettsiaceae—evide chemical similarity On the other hand they are completely different of the viruses In size, the Chlamydozoaceae are of a size comparable t of the Rickettsiaceae (when allowance is made for shape), and are not larger than the largest viruses the *Borreliota* or poxes

Antigenically there are certain resemblances to the Rickettsiaceae tralizing antibodies are hard to produce in high titer and passive prot by serum transfer is not usually highly successful A more striking r blance lies in the possession by all agents of the two groups so far adeq examined of a toxic component different from bacterial exo- or endo-tox from the toxic components described for certain viruses particularly c influenza group There is not space to discuss other features of the an antibody pictures presented by these agents This has been discussed where

Finally one should draw attention to the susceptibility of the Chlan zoaceae to chemotherapeutic agents Among the viruses such suscepti does not exist except for the group of ill-defined pneumotropic agents re sible for a typical viral pneumonia in man, cotton rat pneumonia, and grey pneumonia in mice On the other hand the Rickettsiaceae share with Chlamydozoaceae a susceptibility, of greater or lesser degree to almost the antibiotic and synthetic drugs most effective for antibacterial chemo apy This, it seems to me, must indicate a most fundamental differen metabolism between these agents and the viruses, a gap which, in the pr state of our knowledge must oblige us either to use this group of agents link between viruses and rickettsiae or compel us to separate them from viruses and place them with the rickettsiae I prefer the latter course

Granted that the Chlamydozoaceae belong with the rickettsiae then it

natural to follow the system of classification set up for the latter Thus over a period of years has been the Linnaean system Moshkovsky system for the Chlamydozoaceae (with a few corrections for obvious errors and some additions to include later knowledge) was therefore applicable and the rules of taxonomy made it mandatory to adopt it The classification shown in FIGURE 1 was the result I have no liking for most of the names which are particularly dissonant and clumsy to our ears but this is no reason to discard them

Two further points should be made concerning this classification which is what was published in the 6th edition of Bergey s Manual It will be noted that the coccoid agents first described by Coles as responsible for conjunctivitis in cattle and poultry are classified under the Chlamydozoaceae as a new genus *Colesiota* Formerly they had been regarded as belonging to the Rick

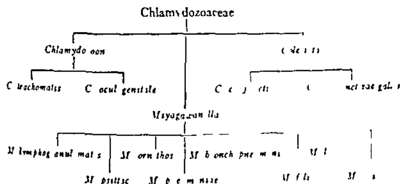


FIGURE 1

ettsiaceae Their reclassification is believed justified not only on morphological grounds but also on the basis of tissue tropisms Predilection for the conjunctival tissues is shown by several of the Chlamydozoaceae including the agents of trachoma and inclusion blennorrhoea in which no other tissues are affected and also those of lymphogranuloma venereum and feline pneumonitis among the *Miyagawanellae*

The other point to be discussed briefly is the problem of classification within the *Miyagawanellae* It will be noted that eight species are listed The right of some of these particularly perhaps *M illinii* to be considered as distinct species may be questioned Further investigation may well show that true species do not exist but that the described differences are only at the strain level Such lack of complete clarity is in my opinion no reason for refusing to undertake classification at this time Such classification should be regarded as an active undertaking open to change at any time that new evidence compels such a change It should indeed serve to point to areas of uncertainty and be an incentive to further investigation

If certain species suggested in FIGURE 1 may disappear in the light of future

knowledge it has become apparent that two new species discovered since the publication of the sixth edition of *Bergey's Manual* must be added These are the agents responsible for salmon disease, and *M bovis* (Baker) the agent of diarrhea in calves[10] and possibly also a prime etiological agent in bovine mastitis

In conclusion the classification of the lymphogranuloma psittacosis group of agents has been discussed Reasons have been advanced for placing the agents with the rickettsiae rather than with the viruses Sufficient knowledge has been accumulated about the various members of the group to make it possible to discuss them both as species, and under the division into three genera Some points of uncertainty remain, but these are not sufficient to argue against classification at this time The classification published in Bergey s Manual has been sufficiently clear and useful to allow two new species to be characterized and named as separate species since the manual was published

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THE NOMENCLATURE AND CLASSIFICATION OF THE POX GROUP OF VIRUSES

By G. John Buddingh

[illegible] State University School of Medicine, New Orleans, La.

Traditionally the pox viruses have been considered to represent a clearly defined group. Edward Jenner's investigations into the nature of the variola vaccinae disclosed the affinity which exists between smallpox and cowpox. The names of Henderson, Bollinger, Guarnieri, Borrel, Paschen, von Prowazek, Lipschutz, [illegible] and Ledingham, to mention but a few, recall researches from which concepts were derived that proved to be basic in the development of knowledge regarding viruses and viral diseases. These classical investigations were concerned with the natural biological phenomenon presented by a group of diverse diseases which have the pox as a common manifestation.

The characteristic papular cutaneous eruption is the predominant feature of several of the diseases produced by the viruses of the pox group. Cutaneous epithelium parasitized either exclusively or during the eruptive stage of the [illegible] process. The proliferative response of susceptible epithelium which comprises the basic constituent of the typical pock is essentially the same in each of the pox diseases. The parasitized cells characteristically contain [illegible] inclusions which have come to be accepted as pathognomonic [illegible] serve to distinguish each from the others. In its own characteristic manner each type of inclusion body is composed essentially of smaller organized particles, the elementary bodies, the infectious units, the virus itself.

Within narrow limits of size and shape there is a remarkable uniformity among the elementary bodies of the pox viruses. They react characteristically [illegible] staining methods, especially the silver precipitation or impregnation method of Morosow.[1] This reaction suggests the presence of physical and/or biochemical features shared in common by the members of the pox group.

The foregoing considerations provided criteria for Goodpasture's proposal to assign a generic and specific nomenclature to members of the pox group.[2] The generic term *Borreliota* was introduced. It recognized Borrel's discovery (in 1904) of the specific granules of fowl pox and applied directly to the elementary bodies. The suffix *iota* signified "smallest particle," and thus the "small particles of Borrel."

The specific term newly devised indicates that the mammalian pox viruses presumably represent modified strains of the same species. Where necessary [illegible] or variety name for viruses of related strains was added. Thus [illegible] nomenclature was proposed: *Borreliota variolae hominis*, *Borreliota* [illegible] *Borreliota* [illegible] *Borreliota* [illegible] *Borreliota* [illegible] and *Borreliota* [illegible]. It was suggested that [illegible] be accepted as the type species.

[illegible] found current acceptance in Bergey's *Determinative* [illegible] Whether or not the term Borreliotaceae should [illegible] Viruses of the Pox Group, including discrete primary or secondary lesions of the [illegible]

knowledge it has become apparent that two new species discovered since the publication of the sixth edition of Bergey's Manual must be added These are the agents responsible for salmon disease, and *M bovis* (Baker) the agent of diarrhea in calves[10] and possibly also a prime etiological agent in bovine mastitis

In conclusion the classification of the lymphogranuloma psittacosis group of agents has been discussed Reasons have been advanced for placing the agents with the rickettsiae rather than with the viruses Sufficient knowledge has been accumulated about the various members of the group to make it possible to discuss them both as species and under the division into three genera Some points of uncertainty remain but these are not sufficient to argue against classification at this time The classification, published in Bergey's Manual has been sufficiently clear and useful to allow two new species to be characterized and named as separate species since the manual was published

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The characteristic papular cutaneous eruption is the predominant feature observed in the diseases produced by the viruses of the pox group. Cutaneous epithelium is parasitized either exclusively or during the eruptive stage of the infectious process. The proliferative response of susceptible epithelium, which comprises the basic constituent of the typical pock, is essentially the same in each of the pox diseases. The parasitized cells characteristically contain intracytoplasmic inclusions, which have come to be accepted as pathognomonic and serve to distinguish each from the others. In its own characteristic manner, each type of inclusion body is composed essentially of smaller organized particles, the elementary bodies, the infectious units, the virus itself.

Within narrow limits of size and shape, there is a remarkable uniformity among the elementary bodies of the pox viruses. They react characteristically to certain staining methods, especially the silver precipitation or impregnation method of Morosow.[1] This reaction suggests the presence of physical and/or biochemical features shared in common by the members of the pox group.

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The specific terminology devised indicates that the mammalian pox viruses presumably represent modified strains of the same species. Where necessary, a subspecific or variety name for viruses of related strains was added. Thus, the following nomenclature was proposed: *Borreliota variolae hominis*, *Borreliota variolae bovis*, *Borreliota variolae equi*, *Borreliota variolae porci*, *Borreliota variolae ovium*, *Borreliota muris*, and *Borreliota avium*. It was suggested that fowl pox virus be accepted as the type species.

This nomenclature has found current acceptance in Bergey's *Determinative Bacteriology* (6th ed., 1949). Whether or not the term Borreliotaceae should designate a more inclusive family of "Viruses of the Pox Group," including diseases characterized in general by discrete primary or secondary lesions of the

nature of macules papules, vesicles or pustules,'[3] is highly debatable The inclusion of some 21 widely divergent human mammalian and avian viral species into a rather loosely designated category imposes *assumptions far be* yond the intent of the original proposal

The use of Borrel s name implied priority of discovery of the elementary bodies in this case those of fowl pox Gordon[4] presented acceptable evidence that Buist, of Edinburgh, made observations in 1886, which indicated that he recognized the elementary bodies of vaccinia and implied their etiological role in the disease The designation *Buistia paschent* was thus proposed for vac cinia virus[5] It would seem that in this case priority should be given to the proposal for the generic nomenclature Goodpasture s classical demonstration of the infectivity of the isolated and *fractionated inclusions of fowl pox*[6] *might* be considered to justify the priority in the designation of the nomenclature for this group of viruses

A definitive classification of the viruses to be included in the pox group should perhaps begin with the genus *Borreliota* as designated by Goodpasture The criteria which were established in assigning the three species to this genus can be applied with good effect for inclusion or exclusion Well recognized attributes of the elementary bodies of variola vaccinia fowl pox and mollus cum contagiosum served to distinguish these viruses as a separate group *These distinctions were (1) morphological (2) the reaction to Morosow's silver* stain (3) the presence in parasitized cells of cytoplasmic inclusions, of which the elementary body constituted the essential component and (4) serological and immunological reactions

The electron microscope has discovered details of morphology which support the validity of the original classification Under the conditions imposed by this technique of visualization the viruses of variola vaccinia, fowl pox and molluscum contagiosum appear to be rectangular and more or less bricklike[7,8,9] By the same means the viruses of ectromelia and infectious myxomatosis appear to have the same shape *Measurements indicate that relatively* narrow dimensional limits characterize these six viruses their combined average range extending from 210 to 264 by 302 to 322 mμ[9]

There is no available report indicating that the virus of ectromelia can be visualized by means of Morosow's stain Vaccinia variola, fowl pox mollus cum contagiosum and infectious myxomatosis react identically to this *method* and are practically indistinguishable from one another on this basis It is quite likely that ectromelia virus possesses the same properties

Varicella and herpes zoster virus also have been shown to be brick shaped *in electron micrographs*[10] These viruses are of a definitely smaller dimen sional magnitude averaging from 197 to 221 mμ They are not visualized by Morosow s stain By means of Paschen's method for staining elementary bodies varicella can be visualized by ordinary microscopy but its evidently smaller size distinguishes it definitely from variola and vaccinia[11] *These vi* ruses like that of *herpes s mplex*, are furthermore *characterized by the fact* that parasitized cells contain intranuclear rather than intracytoplasmic inclu sions The cutaneous eruption which they induce is in several essentials different from that induced by the *pox viruses* The lesions are essentially

vesicular rather than papular and their histological characteristics sharply distinguish them from the pox It appears rather doubtful that a single morphological feature (that of a bricklike shape) should suffice to include varicella and herpes zoster within this group

Little if any difficulty is encountered in assigning different types of viruses to this group on the basis of the intracytoplasmic inclusions that they induce in parasitized susceptible cells This particular attribute common to this group of viruses should be maintained as a fundamental criterion on which a classification is erected Perhaps more than any other attribute it denotes behaviorisms of the parasite in relation to the host cell which are specifically definitive The histological characteristics of the inclusion bodies induced by the pox group are pathognomonic for each species and strain There is sufficient evidence to accept the conclusion that these structures in each case and each in its peculiar way, are essentially composed of elementary bodies representing intracellular aggregations of virus particles Fowl pox cow pox molluscum contagiosum ectromelia vaccinia variola and infectious myxomatosis each meet the cytopathological criterion represented by the intracytoplasmic inclusion body

The question of the specificity and significance of the intranuclear inclusions described as characteristic for variola and so-called paravaccinia, should be kept in suspense for the time being More careful study will be required to arrive at a definite conclusion as to whether or not the intranuclear inclusion of variola actually represents parasitization of the nucleus by the virus a chance infection with an unknown accompanying virus or a degenerative change induced by variola infection The existence of paravaccinia as a definite entity has not been sufficiently substantiated to warrant its serious consideration in this regard

Serological reactions whereby the antigenic components of a microorganism can be more or less strictly defined, present the most satisfactory means for establishing classifications Although they rarely serve to designate the presence of an antigenic component common to an entire genus species interrelationships are often brought out with considerable nicety These circumstances also apply to the pox group Specific serological and immunological differences distinctly separate the various species As yet no cross-immunization or common antigenic components have been found to exist between the species designated as *Borreliota variolae Borreliota mollusci Borreliota avium* and the virus of infectious myxomatosis Their specificity as distinct species in the genus is derived on the basis of the other criteria which have been discussed

Considerable serological evidence has been presented which demonstrates the close affinity between certain members of the mammalian poxes Variola and vaccinia present the classical example of this relationship By means of newer and more exact techniques investigations of recent years have established a firmer basis for the *serological classification* of the mammalian poxes Furthermore they have introduced at least two new subspecies or varieties to this group

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represents a type or variety distinct from variola and from vaccinia Not only are antigenic differences demonstrable but to those familiar with the characteristics of inclusion bodies *definite distinctions are evident*

Ectromelia or mouse pox has been shown by Burnet and Stone[14] and Burnet and Boake[15] to be related serologically to the mammalian poxes Cross-antihemagglutinin and cross neutralization reactions have demonstrated the relationship of this strain to the group Fenner s studies[16] on the natural history of ectromelia have indicated that *this infection serves as a good model* for the study of the pox diseases

The carefully conducted experiments of McCarthy and Downie[15] in which cross neutralization of different strains by means of antisera prepared against each strain was measured by the 'pock counting" technique of Burnet serve as a beginning of the solution of the problem presented by the *interrelation* ships of the mammalian poxes The studies of Horgan and his associates[17] on *the relationships* between various strains of variola and alastrim are quite suggestive of the existence of a variety of antigenic patterns which typify the human pox viruses Continued efforts in this direction should bring out clearer delineations There is at least, sufficient evidence to justify a definite distinction to be made between variola and alsatrim keeping in mind that very likely *each represents groups of strains There are however, insufficient data* available to implicate unreservedly antigenic strain differences to pathogenicity thereby accounting for the numerous clinical varieties of human smallpox

Careful consideration leads to the conclusion that the strains of vaccinia virus generally used in prophylaxis against smallpox should be considered as *a distinct separate subspecies They differ in several respects from the* natural cowpox virus which Downie has recently characterized Exact knowledge regarding the actual origin of most commercial vaccinia strains is not obtainable There is good reason to believe that some of them can be traced back to strains originally isolated by Jenner but there is not sufficient acceptable evidence to prove this without question The considerable variety of empirical methods whereby the vaccinia of commerce has been maintained since Jenner s day, has resulted in the *establishment of what must be regarded as essentially* artificial or at least laboratory strains Presumably they have one attribute in common, in that they are relied upon to induce human vaccinia by which prophylaxis against smallpox is established

This admitted variation in the characteristics of existing strains of vaccinia virus used *throughout* the world for smallpox vaccination emphasizes the desirability for the establishment of criteria that would determine the qualifications of a type ' or ' reference strain *Well controlled and reproducible* laboratory procedures are available for determining *whether or not a given* strain stimulates the production of antibodies capable of reacting *with or neutralizing variola* virus It would be difficult at present to decide whether or not the persistence of a given level of antibody over a given period of time would be indicative of *adequate protection* against variola It is at least obvious that strains propagated in a single tissue of a single host become homogeneous Thus vaccinia propagated through *numerous passages* in the *chorio* allantois over many years becomes highly dermotropic producing a mild

superficial infection in man and in rabbits [18] Such strains presumably do not affect the immune mechanism as profoundly as do those which are more heterogeneous in their infectivity for cells of various types [19 20]

In this regard woeful ignorance exists Only a few suggestions might be hazarded to indicate what a type strain of vaccinia should be like It should be propagated in calves, i.e it should first of all be vaccinia Presumably it should be regularly enhanced by fortification by passage through man and the rabbit It should possess a degree of heterogeneity in that its infectivity is not restricted to epidermal epithelium but should also involve mesodermal fibroblasts There is good reason to believe that it should induce a transitory viremia determined by isolating the virus from the pharynx during the early stages of the induced disease

It is not possible to undertake a consideration of the numerous aspects of this problem in the available space allotted Without considerable carefully planned experimental work it does not seem feasible to establish the criteria that would characterize a type strain of vaccinia The first requirement that must be met is to outline the problem in its numerous aspects This would definitely require a conference of those qualified by experience those concerned with the practical aspects of manufacture and those concerned with the broad implications of preventive medicine

The position of other members of the mammalian pox group for the time being must be accepted as tentative The more recently developed experimental techniques have not been applied to rabbit pox swine pox sheep pox goat pox and horse pox There is good reason to believe that in the event this is done these viruses will fall into the category a strains or subspecies, as originally designated by Goodpasture Their final classification however should await more definitive investigation

Among the bird poxes at least three varieties (fowl pox pigeon pox and canary pox) are recognized Certain distinct differences in pathogenesis and species susceptibility exist between them No definitive work has as yet been done to differentiate them antigenically by serological analysis For the time being these three strains perhaps should be considered as a single species without sharply defined subgroups

The foregoing considerations present a basis for the following enlarged and somewhat revised nomenclature and classification of the viruses of the pox group

Genus Borreliota *Goodpasture*

Viruses of the Typical Pox Diseases of Man and Animals They induce diseases characterized by papular cutaneous eruptions The virus particles appear brick shaped in electron micrographs with dimensions from 210 to 264 by 302 to 32 mμ They are visible by ordinary micro copy in smear preparations stained by Morosow s silver method The virus particles constitute the essential component of the intracytoplasmic inclusions which characterize parasitized cells The generic name recognizes Borrel s original description of the specific granules or elementary bodies of fowl pox The suffix *iota* the Latinized name of the smallest Greek letter signifies smallest particle and hence the small particles of Borrel

The type species is *Borreliota avium* Goodpasture

Key to the species of genus *Borreliota*

(1) *Borreliota variolae* the mammalian pox viruses

Subspecies or strains

(a) *Borreliota variolae hominis major*, classical smallpox

(b) *Borreliota variolae hominis minor*, alastrim

(c) *Borreliota variolae vaccinia* vaccinia

(d) *Borreliota variolae bovis*, naturally occurring cow pox

(e) *Borreliota variolae ectromelia* mouse pox

(f) *Borreliota variolae ovium*, sheep and goat pox

(g) *Borreliota variolae porci* swine pox

(h) *Borreliota variolae equi* horse pox

(2) *Borreliota mollusci* molluscum contagiosum

(3) *Borreliota avium* fowl pox pigeon pox canary pox

(4) *Borreliota myxomatum* infectious myxomatosis of rabbits

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INFLUENZA VIRUS GROUP

By Sir MacFarlane Burnet

The Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia

In discussing the extent of this group and the criteria for differentiation of species within it, we must accept the practical necessity of using strains which have been adapted to laboratory passage, usually in the allantoic cavity of the chick embryo. In the influenza A group at least, the wild virus cannot be maintained and studied in its original state without an inordinate amount of labor.

I shall consider that there are six species with sufficient characteristics in common to justify their being combined in a single group, although subsequent study may demand that two or even three genera be created to contain them. The viruses are influenza A, influenza B, mumps, Newcastle disease of fowls, fowl plague, and influenza C (Francis). The primary reason for grouping them together is that all adapted strains in adequate concentration agglutinate chicken cells, the virus particle itself being the agglutinating agent. Adsorption of the virus to the cell surface is in all cases followed by complete or partial elution by the enzymic action of the virus on the cell receptor. In the case of five of the viruses the substrate can be shown to be of mucoprotein character and the enzymic mechanism of similar nature. Influenza C, on present knowledge, appears to utilize a different substrate.

Other features are:

(1) The virus units are of approximately equal size. All show spherical units and, with the exception of mumps virus, all have been shown to give rise to filamentous forms.

(2) All are readily destroyed by ether and by surface active agents such as sodium desoxycholate.

(3) All multiply in the cavities of the chick embryo, liberating large amounts of virus into the fluid.

Newcastle disease and mumps viruses differ significantly from the others in their reaction with the red cell. Both, under appropriate conditions, are hemolytic and both can combine firmly with the cell surface so that they cannot be detached by immune serum or the receptor destroying enzyme of *Vibrio cholerae*. They also differ in their tissue tropisms, though it must be recognized that high concentrations of Newcastle disease virus produce pulmonary lesions in mice. Although no appreciable virus multiplication occurs, the lesions are produced by what seems to be the same mechanism as is responsible for the initiation of infection by influenza strains in the same situation. For these two viruses a new genus may be needed eventually, but for the time being they should be kept with the others of the group.

A joint discussion with Dr Andrewes and Dr Francis led to our belief that Dr Holmes' name *Tarpeia* might be adopted for the genus. I shall leave the suggestion of specific names to Dr Andrewes.

POSSIBLE CLASSIFICATION OF THE ARTHROPOD BORNE ENCEPHALITIS VIRUSES

By W McD Hammon

Department of Epidemiology and Microbiology Graduate School of Public Health University of Pittsburgh Pittsburgh Pa

Together with an associate in California, Dr William C Reeves, the author introduced to the literature[1] the term 'arthropod borne viral encephalitides now widely used in the classification of neurotropic viruses This term apparently has served a useful purpose, although perhaps only *ad interim* At the International Congress of Microbiologists at Petropolis Brazil in 1950 this group of viruses was one of those selected by the Virus Subcommittee of the International Nomenclature Committee as possibly being ready for more formal classification and the application of the Linnean nomenclature In this paper an attempt will be made to review current knowledge of this group of viruses as it might apply to classification This will be done with considerable brevity, since two eminently qualified scientists will discuss the same subject in this monograph

The present descriptive group title the arthropod borne encephalitis viruses is based on clinical and epidemiological criteria encephalitis and a natural arthropod vector Names applied to individual viral agents in the group are generally geographical and, in some instances include the name of the first mammalian host recognized to be affected by the disease (the horse) even though by present knowledge it is not usually the principal vertebrate host in the biological cycle of the virus Thus the host name does not appear to be particularly appropriate at present Examples of these types of names are St Louis and Japanese encephalitis (geographical) and western eastern and Venezuelan equine encephalomyelitis (geographical and host name) In some other possible members of the group not yet known to produce encephalitis through natural means of infection the generic name of the arthropod host from which the virus was first isolated is used instead of a geographical name *i e* *Anopheles* A *Anopheles* B and *Wyeomyia* viruses[2] *etc* Louping ill virus an exception bears the name of the disease it produces in sheep and to conform to pattern, might have been named Scotch ovine encephalitis or encephalomyelitis

Unfortunately the exact extent of the arthropod borne encephalitis group cannot be delineated at present since, for many viruses possibly belonging in the group certain fundamental information is completely lacking such as the nature of the clinical disease the agent may produce in nature if any the vertebrate host or hosts included in the natural infection or disease spectrum the arthropod vector or whether such a vector exists The following groups of agents possibly belonging in the arthropod borne viral encephalitis classification, are suggested for consideration

(1) Those definitely acceptable as arthropod borne encephalitis viruses since proof or strong probability exists of clinical encephalitis acquired naturally through an arthropod vector *i e* western eastern and Venezuelan equine

St Louis Japanese Russian louping ill and California types We are writing a series of papers at this moment[8] which we believe furnishes adequate evidence to support the inclusion of the California virus in this group It should be noted that all of these, with the exception of louping ill carry geographical names

(2) Those possibly belonging in the group but about which adequate knowledge is lacking for definite classification A very large number of viruses fall into this uncertain classification These can be considered in five general groups

(a) Arthropod-derived viruses that produce encephalitis in certain laboratory animals but whose natural host and type of disease produced in them in nature are unknown i e Semliki Forest[6] Bunyamwera[7] Ntaya[8] etc These also carry geographical names

(b) Those related serologically to the St Louis Japanese West Nile group with known arthropod vectors and producing encephalitis in laboratory animals but normally not producing encephalitis in man i e yellow fever and dengue[9] These were named long before their epidemiology was understood and carry clinically descriptive names

(c) A group of viruses of small size and ether resistant all closely related or identical immunologically[10 11] These viruses were isolated from primates, marsupials probably from rodents and twice from mosquitoes One human infection was encephalitic in type and encephalomyelitis is regularly produced in a number of laboratory animals Rodents are presumably the principal host at least in this country[12] This virus complex is known as the Columbia SK MM-encephalomyocarditis Mengo group These will be considered separately by other authors in this monograph and probably do not belong in this group but being encephalitogenic and having been isolated from mosquitoes they cannot be excluded from preliminary consideration

(d) We must give some consideration for purposes of proper classification to a miscellaneous group of animal viruses that may regularly or under certain conditions produce encephalitis in laboratory animals and are apparently transmitted naturally by arthropods They are neurotropic although not in their natural hosts and are arthropod transmitted Two examples of this group are Rift Valley fever and Colorado tick fever When man is infected with these viruses a denguelike disease occurs So far no immunologic relation is recognized between these and the forms which can be definitely classified in the arthropod borne group These viruses also carry geographical names in conformity with the general pattern

(e) Viruses of another group requiring consideration cause encephalitis in certain laboratory animals and have been isolated from nonencephalitic natural human infections but, although suspected are not yet known to be arthropod borne One of these West Nile virus[3] is related immunologically to the arthropod borne Japanese St Louis group[14] and has been experimentally transmitted by mosquitoes[15] Another virus at present belonging in this ill-defined group is Bwamba fever virus[6] These again are geographical names

There are still other viruses with definite neurotropism but certainly not dependent on arthropod transmission Should this factor alone exclude them from belonging in the same genus or subclassification of some type? Or does neurotropism assume adequate significance to serve as a basis for binomial classification? Should the present clinico-epidemiological concept arthropod borne encephalitis serve as a basis for classification at all? What weight should be placed on immunologic groupings such as dengue yellow fever and St Louis Japanese West Nile groups? Finally, on what criterion or group of criteria should we attempt further classification? The following list, in order of preference or weight has been suggested by the international subcommittee and discussed previously in some detail in a paper by Andrewes [17]

(1) Morphology and methods of reproduction
(2) Chemical composition and physical properties
(3) Immunological properties
(4) Susceptibility to physical and chemical agents
(5) Natural methods of transmission,
(6) Host, tissue and cell tropisms
(7) Pathology including inclusion body formation
(8) Symptomatology

Unfortunately we have at present so little information on most of these points that further attempts to formalize the classification of this group seem unwise at this time For example certain of the viruses are immunologically related and this permits some grouping It also involves, however inclusion of yellow fever and dengue with St Louis and Japanese B and also West Nile which so far is known to produce encephalitis only in laboratory animals and is not known to be arthropod borne The Columbia SK MM EMC Mengo group are of smaller size than most of the others and have a recognized greater resistance to certain chemicals to heat to lyophilization and to pH changes which strongly suggests they should not be included with the others

The sizes of the viruses we have been considering among those most likely to belong in this group vary greatly, ranging from Japanese encephalitis 18 mμ [18] as calculated by one worker to California virus which we estimate to be between 60 and 125 mμ [4] Several of these viruses have not yet been visualized by the electron microscope and size estimations either have not been made by any method or estimations from different laboratories fail to show reasonable correlation

The methods of reproduction of these viruses are essentially unknown

Certainly little or nothing is known about chemical composition of many members of the group and those that have been studied require further investigation after better techniques become available

Studies on physical properties aside from morphology are also completely inadequate for most of the viruses to be of value in classification as are studies on susceptibility to physical and chemical agents

Natural methods of transmission have been discussed and inadequacies in these data are obvious although encouraging progress is being made

Host tropisms form a field which has received a comparatively large amount of study for some members of the group and we find great variation even be

tween closely related agents by other criteria This information therefore is of little aid to classification Upon what host should we place greatest emphasis from the standpoint of classification? Man appears to be an unimportant and accidental or aberrant host for most of these viruses from St Louis and western equine encephalitis to jungle yellow fever Possibly he is completely resistant to infection with some of the viruses listed Horse also are unimportant biological hosts even for some of those named equine encephalomyelitis viruses but this mammal may be an important host for other viruses While birds appear to be the most important biological host for a number of these viruses they may nevertheless not be susceptible to any clinical disease resulting from natural infection as in western equine and St Louis infections[19] Rodents sheep or monkeys may be the host of greatest biologic importance in others of the group Chick embryos and mice appear to be susceptible to infection with all but there are great differences in susceptibility and special adaptation procedures may be required before some viruses can be propagated in these animals According to routes of inoculation susceptibility of animals also differs markedly even between closely related viruses in the group

Regarding tissue tropisms these viruses vary from pantropism to what appears to be relatively strict neurotropism

The pathology in the brains of laboratory animals is rather strikingly similar with destructive and inflammatory lesions and no significant inclusion bodies except for yellow fever

Symptomatology varies widely depending on the host and there is nothing pathognomonic for the group

Thus except for (1) immunologic relationship (which with very few exceptions, make the total group under consideration completely unrelated) and (2) the epidemiological-clinical grouping (which unites the group with certain limitations and uncertainties) available knowledge does not permit further intelligent classification

In conclusion although to accomplish something more constructive would have given great satisfaction it appears that the following statements best describe the present status

(1) Present classification and nomenclature although imperfect and incomplete do not in themselves lead to confusion encourage multiplicity of names or lead to inept terminology Most of the names have been applied in a relatively systematic way on the basis of available knowledge

(2) Much more specific knowledge than is available at present would be necessary before improvement or change could be made in classification

(3) Attempting classification now into family genus and species would simply make obvious very essential future changes more difficult to effect and cause unnecessary confusion and difficulty during the apparently ill timed transition

(4) Present general group classification serves a useful clinical biologic epidemiologic purpose and uncertain fringe groups will find their proper places (in or out) with time

(5) Further intensive epidemiologic field and fundamental laboratory study

is essential to discover suitable criteria for a rational and basic concept of better classification

(6) The classification suggested for these viruses by *Holmes appears to have* been very premature and should not be used

Discussion

Now after having finished my formal and rather brief presentation I should like to indulge in a little less formal discussion

Previous authors have emphasized repeatedly that any classification should be based primarily on fundamental properties of the viruses themselves such as morphology chemical composition and physical and immunological properties and *not* on the diseases they produce or the hosts that happen to be susceptible I believe it will be obvious to all of you by now that those viruses I have been requested to consider are grouped together because (1) they produce one type of disease encephalitis (2) they happen to have an arthropod as one of a series of hosts and (3) possibly also because man is occasionally a victimized host If we consider these known characteristics by which this group was established as factors of minimal importance for suitable binomial classification, do we know enough about the virus particles, themselves to *entitle us to classify them together and to exclude others? Most certainly*, we do not know enough to place them in suitable relation to each other in order to classify them with any certainty by genus We can of course, pretend to have knowledge we do not have and set up a hypothetical idol with clay feet to be gilded with latinized codification Let us remember, however, that part *of this will remain as a permanent record of our presumption of* insight into the evolution of these organisms This classification would be set up with the full knowledge that it would crumble time and again as scientific knowledge replaced guess work We have been assured repeatedly by our systematists that this binomial system is flexible and that changes are to be expected and can readily be effected Out of necessity many scientists have been willing to indulge repeatedly in this type of pretentious display of a knowledge of phylogeny Binomial classification became necessary *in order to bring some* organization out of chaos where literally thousands of common names existed for the same plant insect, or bird of more or less world wide distribution which was known described, and associated with local names by scientists and lay amateurs alike What necessity, however is there for such pretension in the field of arthropod borne *encephalitis viruses* which can be observed only with an electron microscope and are handled by only a few hundred specially trained scientists at most in a much smaller number *of scientific* institutions where there is access to international scientific literature? To date there is in most instances only one common name for each of the viruses *in the group after* suitable translation of a geographic name The progress of science in this field does not appear to demand any departure from our usual scientific caution to build gilded clay *footed images to* haunt us and the following generations in future reviews of the literature Do we pause then to consider definitive classification to be undertaken immediately *and* because of a clamor from our own ranks try to keep up with the Joneses?' I do not

believe this to be the case among workers in this specific group Instead we are considering this possible need at the request of a few of our animal virus brethren who themsleves are being pressed to fall in line by groups who actually do find their field in a chaos of common names and who quite possibly need binomial classification at this time In so far as my field is concerned the question appears to me to be Will binomial classification help to reduce confusion in th group at this time *i e* is it needed?' and not Is this group ready for such classification? In either case my an ver at this moment is No but the answer is much more emphatic to the first question I see no reason to go through the action of putting out a fire in my yard where none exists just because my neighbor is engaged in putting out a real one in his yard

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ON THE NOMENCLATURE AND CLASSIFICATION OF ARTHROPOD BORNE ENCEPHALITIDES

By Pierre Lepine
Virus Research Division Pasteur Institute Paris France

During the last few years the problem of the nomenclature of the viruses has been brought up frequently and from many different sources It came to a head and crystallized with regard to the suitability of making use of the Linnean system of classification by binomial denomination We have to remember that classification and denomination are, as concepts quite distinct although they are closely linked and related The question of denomination is after all of secondary importance if the usual names applied to the viruses and if the terms by which they are commonly designated are sufficiently clear not to allow for any confusion Nevertheless new names have been proposed for rechristening the known viruses These have been accepted by some and rejected by others sometimes even with indignation Thus indirectly they have awakened the interest of academic bodies in the classification of the viruses resulting in the conference on which this monograph is based

The changing of the original names for Latin names with binomial keys though of interest, would be justified only if based on the preliminary establishment of a logical classification *i e* if one could delimit exactly among the different genera each one being clearly distinguished from every other one so that it would be possible to recognize the related individual species within the established framework One sees therefore, that the choice of names is in itself, a futile question and that it should be raised only after complete agreement has been reached with regard to the basic principles of a classification

Ever since Linnaeus succeeded in introducing a logical order (culminating in a classification) in the description of the various botanical species and later of those of the animal kingdom all attempts at classification in biology have made use of the principles set forth by him

If one examines the work of Linnaeus one perceives that his classification rests essentially on phylogeny This means that starting from paleontology which deals with the reconstruction of the extinct species of the animal and vegetable kingdoms it retraces the genealogical tree of existing species and in this way establishes the groupings and the connections of the individual species with regard to their present time relationship and to their original common ancestry

Consequently in order to establish a Linnean classification *i e* to group families of living beings and establish their relationships it is necessary to be able to distinguish the primitive species from those more evolved and thus to be in a position to recognize not only the relationships between the present groups but also to determine which amongst them preceded the others chronologically, so that the successive ramifications derived from the original stem may be traced

If for the higher species of the plant and animal kingdoms such an under

taking is relatively easy in that use is made of the precise knowledge which is gathered through the study of evolution the problem is quite different and much more difficult when one has to deal with the more elementary beings such as bacteria

A recent paper by Winogradsky[1] rightly called attention to the difficulty which exists even for the bacteria which we know best to establish biotypes or type species and to recognize the ecological types derived from the fundamental type species in order to establish a natural classification

If, from the very beginning one encounters such problems in the study of bacteria and monocellular microorganisms (some of which have been cultured and used in experiments in the laboratory for nearly a century allowing us to follow them during a very great number of generations and to determine their characteristics at leisure) what then must be the difficulties perhaps insuperable that we must overcome in attempting to establish a system of classification of the viruses? In addition when speaking of viruses we must not forget the incessant plasticity of their fundamental characteristic and the fact that they most often appear to us as a scratch collection of unmatched species rather than as a display of well-ordered family groups

Just plain common sense however is sufficient to recognize amongst the several hundred viruses in which we are interested in particular the pathogenic viruses affecting man those agents exhibiting a sufficiently great analogy (e g in their pathogenic power their tissular affinities their physical and morphological characteristics the pathological lesions which they cause and finally, in the immunological reactions which they induce) to be considered as being different members of the same group or of the same family

It was for this reason that the commission which met in Petropolis Brazil in 1950 on the occasion of the Fifth International Congress of Microbiology deemed it opportune that an attempt be made to classify certain groups of viruses and that the results of these trials should be put on the agenda of the next Congress scheduled to take place in Rome in 1953 in order that they may serve as a basis for discussion

Amongst the groups of viruses to be considered the neurotropic viruses of the arthropod borne encephalitides have been retained As a matter of fact during these last few years the research work done on the neurotropic viruses has brought to light the ever increasing importance of a certain number of these viruses which are mostly encountered in the hot temperate zones and which are the agents of encephalites of which the pathogenic and clinical individualities have progressively been untangled All these encephalites some of which have been observed for a long time while others have been recognized more recently present the following characteristics in common

(1) The diseases present a seasonal character with an incidence more marked for the rural or semi rural than for the urban population

(2) The illness is initiated by a febrile phase during the course of which there is a viremia Amongst a number of the infected persons it does not pass beyond this stage and the neurotropism of the virus remains latent

(3) Following the disease with or without symptoms convalescent sera show specific antibodies allowing a retrospective diagnosis

(4) The virus is transmitted by one or several arthropods mosquitoes ticks or mites, the epidemiology of the disease being conditioned by the particular circumstances *of the life-cycle and infection* of the insect

(5) The virus is propagated according to a cycle which is *sometimes* complicated and in which the vector insect plays an intermediary role between the natural reservoirs (rodents and birds) and the susceptible animal (horse or cattle) The *intervention of man into this cycle is accidental* being at times the result of circumstances disturbing the normal cycle of transmission such as the substitution of one species of mosquito by another following soil irrigation or the like

(6) The virus has an average *particle size of 15–30 mμ It is easily grown* in the embryonic chicken tissues The susceptible laboratory animal is the mouse which presents an encephalitis after intercerebral inoculation The *pathology shows disseminated encephalitic lesions* with more or less marked *pycnotic lesions of the cells of the hippocampus major* but *without nuclear or* protoplasmic specific inclusions

To sum up, these arthropod borne encephalites which one may also qualify as seasonal form a vast group which appears to be coherent and suitable for classification

The world wide extension of these encephalites and the particular characteristics they show on the different continents allow their provisional placing into four main geographical groups These are the *American group* the best known until now the *Far Eastern group*, represented especially by the Japanese B encephalitis the *Eurasiatic group* which spreads from Siberia to Scotland and lastly the *African group* the study of which has been started only recently

Without the need of recalling the history of the research work done on the *encephalitides it is easy to recognize the following principal* viruses in the groups described above according to the different diseases which they cause

(1) *American Group* (a) Western equine encephalomyelitis (b) Eastern equine encephalomyelitis (c) Venezuelan equine encephalomyelitis (d) Argentinian equine encephalomyelitis (if this must be separated from the Venezuelan type) and (e) *encephalitis of Saint Louis*

(2) *Far Eastern Group* Type B encephalitis of Japan to which must be connected the encephalitis of Manchuria and apparently also the Australian X disease as well as the disease caused by the recently *recognized* Murray Valley virus *As a matter of fact* the vast territory of this encephalitis stretches far beyond the islands of the Japanese Archipelago since it includes the northern coast of Australia the western islands of the Pacific the *northern and eastern areas of Manchuria and the far-eastern* part of Siberia

(3) *Eurasiatic Group* (a) Russian spring summer encephalitis, the area of which becomes intermingled on its eastern boundary with that of the Japanese B encephalitis while the western limit of this *Eurasiatic group* is represented *by the encephalitis of Moravia*[3] and probably by the encephalitis of Pfalz[4] the nature of which is still indeterminate and (b) The louping ill of Scotland which seems to be spreading throughout the rest of the British Isles[4,5,6,7] but which has not been met *so far* on the European continent

(4) *African Group* It appears to us at the present time at least to have less

homogeneity It includes togethe with authentic encephalitogenic viruses other viruses the neurotropic affinity of which was discovered secondarily or is man fested in laboratory animals only

A typical example of our kno led_e concerning these last named viruses is shown by the findings of the research workers of the International Health Division of the Rockefeller Foundation at the laboratory of Entebbe in Uganda Africa They were eeking to find the virus of the jungle yellow fever among the mosquitoes and ended up by isolating the Mengo virus [8] the pathogenic role of which for man was discovered by chance as a result of a laboratory contamination In a somewhat similar way the viruses of Bwamba Fever Semliki forest Bunyamwera *etc* and a virus to which we shall have to refer again the West Nile virus [9] were isolated from mosquitoe

In addition to the viruses already characterized a few strains of viruses have been isolated in the Belgian Congo or in French Equatorial Africa under different terms or names or starting from cases labeled as poliomyelitis transmissible to rodents These are actually being studied but the findings are as yet unpublished It seems that everal amongst them are antigenically related to certain of the already recognized viruses but that others exhibit certain different characteristics, which do not permit their classification at the present time

This diagrammatic view of the distribution of the viruses of the encephalitides should be understood as a simplified table of our present day knowledge It is only a rough draft which one might make use of as a basis for classification

If we seek to elucidate this problem and to narro it down by an immunological study of the definition of the species or the groups we note the difficulties that immediately confront us

With regard to the American viruses amongst which the differences appear sharply at fir t ight the discovery of a grandparent virus [10] raises the presently unsolved problem of the relationship of the Saint Louis encephalitis to the Western and Eastern equine encephalomyelites Although still not clearly identified the existence of certain encephalomyelites in the equatorial zone (notably those in French Guinea) [11] and some of the isolated strains of Brazil and Columbia for instance the Ilheus virus [12] which later presented surprising immunological relationship with the African and Far Eastern viruses [13] opens the question of the interrelationship of these viruses

Now there is the question of the relationship which has just been established on the basis of cros ed immunity betw en the virus of the Saint Louis encephalitis and that of the epidemic kerato conjuntivitis [1] [14]

Concerning the Eurasiatic group the complete crossed immunity between the louping ill and the Russian spring summer encephalitis is well known since the work of Casals and Web ter [15] Nevertheless neither the clinical characteristics nor the epidemiological data concerning these two viruses permit us to consider them as being one and the same

It was especially the study of the African encephalitis group and the unexpected results obtained therefrom that have radically changed our notions upon which any con truction would have been attempted It is not necessary to recall that the Mengo virus has caused in man as well as in the animal a complete cross immunity with the E M C virus of the Chimpanzee[17] and of the

rat,[18] with a poliomyelitislike disease that attacked the American soldiers in 1945 and 1946 in the Philippines [19] and finally with the MM strain of the mouse poliomyelitis [20] All these viruses *belong manifestly* to a different group from that of the encephalitides from which we should therefore withdraw the Mengo virus

On many occasions the West Nile virus has been found in Africa among individuals showing absolutely no pathological condition and notably in Egypt by Melnick and Paul[21] at the time that they were *doing research on* polio antibodies in healthy children This virus must therefore be considered as being much more widely spread than heretofore suspected

We *are completely* unaware of the significance of the West Nile antibodies encountered outside Africa [20] but Casals showed in 1944 [22] by complement fixation tests that mice vaccinated against the Japanese B encephalitis exhibited at the same time but to a lesser degree antibodies against the Saint Louis encephalitis, against the West Nile virus and against the Ilheus virus

These facts tend to blur the limits of *the groups and species* we have endeavored to recognize The confusion increases still more if we take into account *the relationships noted by some authors like* Meiklejohn or Sabin (still unpublished) between the above mentioned viruses and the viruses such as those of Dengue and Yellow Fever of which there can now be no question of their being included in the true encephalitides

In conclusion as we have tried to show,[4] there exists around the world in the hot temperate zone a continuous belt of seasonal encephalitides transmitted *by the invertebrates These encephalitides form a* vast *family of* afflictions the territories of which are more or less interlocked Although they often appear as having a complete clinical and epidemiological autonomy they are none the less linked by variable degrees of antigenic power and sometimes by a complete cross-immunity

It is easy to suppose that the remote origin (and for certain ones the actual reservoir) of these viruses is to be found in the tropical zone

The transmission by arthropods and more particularly by the ticks the presence *of immunologically related diseases symmetrically* disposed in relation to the equator (for example the case of the American equine encephalomyelitis) the fact that the least differentiated viruses or those presenting the wid st spectrum of antigenic relationships (the case of the virus Mengo West Nile Ilheus *etc*) are met with in the tropics permit assigning to the seasonal encephalites of the temperate regions a tropical origin *which is far* from one of the least interesting of the aspects of the problem they give rise to

These considerations are of a fundamental interest for the epidemiology, the immunology and the *basic study of the viruses under consideration* but in our opinion they make premature all attempts to classify this group of viruses logically

The framework which we might make use of today *for classifying* viruses on immunological and other grounds is *totally different* from that to be expected a few years hence just as it has been modified today as compared to a few years ago At the present time the progress of research is advancing so

rapidly that one can without any fear of contradiction affirm that any attemp to classify too rigidly, on the ground that there is a genealogy of the viruses and a relation hip between them would only lead to radical changes in a ver short time

No matter how interesting the question may be no matter how logical it may seem to establish a cla sification of the viru es of the encephalites our presen inability to delimit the group and to clearly separate the different specie compels me to ask that the establishment of a classification should be adjourned to some time in the future

In the field of virus research we are till at the stage of explorers entering virgin forest which offers to our amazement the discovery of plants and flowers belonging to new varieties and unknown species Let us not demand of ou research workers the cold austerity and the unchangeable frames of the bot anists who work upon plants and flowers long dried between the pages of herbarium

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RELATIONSHIPS BETWEEN ARTHROPOD BORNE VIRUSES BASED ON ANTIGENIC ANALYSIS GROWTH REQUIREMENTS, AND SELECTIVE BIOCHEMICAL INACTIVATION

By Albert B Sabin

The Children s Hospital Research Foundation Department of Pediatrics University of Cincinnati Cincinnati Ohio

Although I hesitate to become involved in the taxonomy of viruses, I shall present a summary of the relationships within a specific group of viruses which may serve as a basis for classification into family, tribe, genus, and species I shall begin by regarding all animal viruses that are arthropod borne in nature as belonging to a single family "Arthropod borne' is used here in the sense that the virus increases in amount after a suitable incubation period in the arthropod host This definition is intended to exclude viruses that may be transferred only passively by arthropods such as trachoma, which is transferred by flies From this family, I shall next select those viruses that by gradocol membrane filtration, have been found to have a size of 15 to 25 mμ and regard them as constituting a single tribe within this family Using size as one criterion for the classification of viruses is in a sense comparable to the use of morphology in the classification of bacteria

Under natural conditions some of the viruses within this tribe are associated with systemic diseases *e g*, yellow fever, dengue, and West Nile fever while others are chiefly noted for the encephalitis they produce *e g* Japanese B (Jap B) St Louis (SLE), Russian Spring Summer (RSSE) louping ill western equine (WEE) eastern equine (EEE), and Venezuelan equine encephalitis (VEE) After intracerebral inoculation however all of these viruses can multiply in the neurones of the mouse and to a varying extent, also in those of the *Rhesus* monkey Antigenic analysis, growth requirements and biochemical reactions have been commonly used for bacterial classification, and I shall continue this exercise in taxonomy by showing how similar or related procedures can be used to subdivide this tribe of viruses into at least two genera

Antigenic Analysis The ten viruses tentatively included in this tribe are all sufficiently distinct antigenically to permit specific identification The only exception to this generalization may be found in the case of the RSSE and louping ill viruses which are so closely related that differentiation may be possible only by quantitative cross immunity tests Seven of the ten viruses in this group have been found to be antigenically related yellow fever dengue West Nile Jap B SLE, louping ill and RSSE For some of these seven viruses the antigenic relationships have been demonstrated by means of neutralizing complement fixing and hemagglutination inhibiting antibodies and for others only by complement fixing antibodies The relationships vary in extent and may be described as major, minor reciprocal or unilateral The minor relationships can be demonstrated with the sera of some animals more readily than with those of other animals of the same species at certain times after the antigenic stimulus but not at other times and with concentrated C F antigens but not with others of lesser potency Apparently all seven viruse

do not possess the same antigens but are linked together by a chain of common antigens Thus the yellow fever dengue West Nile and Jap B viruses possess a common antigen most readily demonstrable by the complement fixation test with concentrated antigens and special sera This common antigen was not found in the strain of SLE tested This SLE virus however is linked to the group by possessing a common antigen with the West Nile and Jap B viruses The louping ill virus which is very closely related to if not identical with the RSSE virus has been reported to possess a *minor* relationship with the West Nile virus No antigenic link has been found between the WEE EEE VEE and the seven viruses just mentioned Although the possibility of a minor antigenic link between the WEE EEE and VEE viruses has not as yet been investigated thoroughly it is clear that these three viruses stand apart from the other seven

Growth Requirements The factors required for viral multiplication, whether they be specific substances enzyme systems or metabolic pathways are supplied by the host Accordingly the pathogenicity of a virus or its host range reflects its growth requirement It is also known that there may be a gradation in the growth factors supplied to viruses by various hosts Some hosts are completely unsuitable and permit no multiplication of virus others appear to be refractory but actually permit the virus to multiply at a low level which is insufficient to kill or injure the host while still others supply an optimum medium for viral multiplication resulting in the severe damage to the host which is the usual basis for including a given host in the pathogenic spectrum of a virus The high pathogenicity for the nervous system of guinea pigs and rabbits uniformly exhibited by the WEE EEE and VEE viruses serves to separate them from the seven other viruses in this group which are not pathogenic for these animal in the usual sense although they may multiply to a limited extent I have recently discovered a genetic factor in the PRI strain of mice which specifically inhibits the multiplication of the seven viruses mentioned but not of the WEE EEE and VEE viruses or of a large number of others tested Extensive tests with the 17 D strain of yellow fever on F_1 F_2 and various types of backcross progeny have yielded data which indicate that the inheritance of this multiplication regulating factor is Mendelian in character There is still another way in which it can be shown that a growth factor which is required by the members of this group of seven viruses is not essential for the WEE, EEE and VEE group As albino rats increase in age from seven to 21 days they lose their susceptibility to intracerebral infection with the SLE and Jap B viruses but not with the WEE EEE and VEE viruses It has been shown that the SLE virus still multiplies in the brain of the resistant older rats but at a 1000-times lower level than in the brain of the seven day-old susceptible rats Mature rats are also resistant to infection with the yellow fever dengue West Nile louping ill and RSSE viruses

Selective Biochemical Inactivation The biochemical reactions that are useful in the classification of bacteria are functions of the microorganisms or their products I wish to cite here a biochemical reaction that although not a function of the virus or of its growth products can destroy selectively the viruses belonging to the group which includes the viruses of yellow fever Jap

B *etc*, but not the WEE virus During the course of a study on the anti poliomyelitic properties of human milk we discovered a large variety of anti viral factors which are secreted at various times in human milk but are not detectable in the serum of the individual Among these antiviral factors was one which after incubation with the viral suspensions at 37°C, destroyed large amounts of the Jap B SLE West Nile dengue and yellow fever viruses but was without effect on the WEE and certain other viruses tested Still another antiviral factor in human milk associated with the lipid fraction and resistant to heating at 100°C for 30 minutes, can destroy the WEE as well as the Jap B yellow fever group of viruses

Thus by antigenic analysis, by the requirement of certain specific growth factors and by selective biochemical inactivation the viruses of yellow fever dengue West Nile Jap B, SLE louping ill and RSSE appear to belong to gether in a single genus despite the fact that they cause different diseases, are transmitted by different arthropod vectors and occur in widely separated parts of the world With the possible exception of the louping ill and RSSE viruses, each of the other viruses in this group can be identified easily as be longing to a distinct species by a variety of other criteria most important of which is antigenic constitution Differences in pathogenic spectrum host range arthropod vectors and properties of hemagglutinins can also be demon strated for each species Similarly, one may regard the WEE EEE and VEE viruses as distinct species of another genus

Now it will be rightfully asked by some that if we can supply criteria for classifying certain viruses into families, tribes genera and species should we not also supply an internationally acceptable, scientific nomenclature? At this point however I must beg to get off the train because I think that I already have gone far past my station

THE COXSACKIE VIRUS GROUP

By Gilbert Dalldorf

Division of Laboratories and Research, New York State Department of Health, Albany, N Y

It seems to me that doubtless we must have more facts before we can hope to devise an enduring taxonomy for the Coxsackie viruses but that without a provisional classification we are likely to waste a good deal of effort and be delayed in assaying their role in nature We need a uniform nomenclature to understand one another

The name 'Coxsackie' has served well It is a distinctive word that Westerners at any rate pronounce without trouble There is nothing in the literature with which to confuse it It is already well established It does not pretend to describe the diseases that the Coxsackie viruses may induce or hypothetical lesions no one has yet seen I suggest we keep the name for a genus of viruses

The definition we originally proposed so far has withstood the challenge of subsequent observations The Coxsackie viruses are selectively pathogenic for immature mice and hamsters inducing in them necrosis of the skeletal muscles with or without lesions of the brain and fat tissues Strains are being encountered that are less selective for suckling mice than were the original types but as yet there is no record of freshly isolated strains that are pathogenic for adult animals The facts that certain strains may be adapted to older animals that older animals are more susceptible following cortisone treatment and that limited organic lesion may be induced in older mice by certain strains do not it seems to me seriously challenge the propriety of the definition They only show how much of an alchemist the modern virologist has become So far as the record goes age susceptibility plus the morbid response in test animals still stand as a serviceable definition

It is well known of course that immature mice are much more susceptible than adult ones to many viruses but I know of no virus other than those producing Coxsackie lesions that is pathogenic only for young animals and so far of none that has as striking an age selectivity It may well be that there will be others but they are still to be described In this connection it may be noted that the lesion of fat has never been observed in other circumstances It may be quite specific

Subdivision of the Coxsackie group seems to me to be both desirable and sensible We have long suspected that the distinction between those strains that produce generalized muscle necrosis and those that produce scattered muscle necrosis plus encephalomalacia and lesions of the fat pads might prove to be of practical importance Anyone who has worked with both varieties knows that the behavior of the sick mice is quite different Visitors to our animal quarters who have known only one kind have no difficulty in deciding whether their strains are A s or B s once they see the two side by side The two groups differ in other respects The Group A strains are easily isolated while the Group B strains are troublesome at times Sometimes the latter are

difficult to adapt to mice sufficiently to permit quantitative testing Group-A strains are more easily adapted More important, there is now substantial evidence that herpangina is associated with Group-A and pleurodynia with Group-B infection, and I think we are justified in incorporating these facts into our classification They may be but the first such associations to be made In both instances the clinical pictures are unusually distinctive

TABLE 1
CLASSIFICATION OF THE COXSACKIE VIRUSES

Group	Type	Representative strain		Strains from other laboratories
		Name	Number	
A	1	T T	48249	Yale–Easton (2) B-J[b]
	2	Fl	49190	
	3	J Oe	49191	
	4	(Howitt)	50746	Minnesota Sy-1 Sy-2[d] Yale–Texas Yale–High Point Dallas M-B[f]
	5	Polk (Howitt)	5128	
	6	C G	5011	
	7	W P	50140	
	8	C D	5010	
	9	P B	50546	
	10	N K	50548	
B	1	P O	49683	Yale–Connecticut 5[e]
	2	Ohio (Red) Melnick[h]	50207	
	3	Nancy Melnick[i]	50531	Washington-518[j]
	4	J V B	51196	Powers[k]

Type Culture Collection number Division of Laboratories and Research New York State Department of Health
[a] MELNICK, J L & A S KAPLAN 1950 Proc Soc Exptl Biol Med 74 812
[b] ROWEN J W & J V IRONS 1950 Texas Reports on Biology and Med e. 8 367
[c] HOWITT B F & U R BENEFIELD 1950 Proc Soc Exptl Biol Med 73 90
[d] SLATER E A & J T SYVERTON 1950 Proc Soc Exptl Biol. Med 74 509
[e] MELNICK, J L 1950 Bull N Y Acad. Med 26 342
[f] SULKIN S E et al 1950 Proc Soc Exptl Biol. Med 73 340
[g] CURNEN E C. et al 1949 J Am Med Assoc 141 894
[h] MELNICK J L et al 1950 J Exptl. Med 91: 185
[i] MELNICK J L & N LEDINKO 1950 J Exptl. Med 92 463
[j] LAZARUS A S E A JOHNSTON & J E GALBRAITH 1952 Am J Pub Health 42 20
[k] CHEEVER F S J B DANIELS & E F HENSEY 1950 J Exptl Med 92 153

Other symptoms that may be due to Coxsackie virus infection seem to be less distinctive and more difficult to relate to the virus

It has interested me to see how differently various workers have approached the problem of causal relationship It is, after all, the old problem for which Koch recommended four criteria known as Koch's postulates Unfortunately Koch's postulates are not all applicable to virus diseases When Rivers discussed this problem some years ago he emphasized the value of a rising antibody titer coincidental with recovery This has frequently been demonstrable of Coxsackie virus infections but may be inconclusive For example the original cases were infected with both poliomyelitis and Coxsackie viruses, and

Melnick found patients who were not only simultaneously infected with both but also had increasing antibodies for both Doctor Huebner and his associates have met this problem by statistical methods emphasizing the importance of regularity of association of infection and disease among cases separated by time and place This is a critical application of Koch s first postulate

In his famous lecture Koch spoke of another criterion that was not incorporated in the four postulates It was the special significance of an association of the suspected pathogen with the characteristic lesion The illustration he used was that typhoid bacilli isolated from the spleen p oved more than typhoid bacilli isolated from the feces I hope that eventually we shall find evidence of this kind for certain Coxsackie viruses It may be most significant

Whatever the outcome we are justified in distinguishing the two groups We should keep our eyes open for other group We have not yet encountered strains that were not readily grouped

We have identified serologic types by Arabic numerals rather than by names following the recommendation of the International Bacteriological Nomenclature Committee Group A Type 4 one of the common types has been found in Texas North Carolina Minnesota and New York Doctor Gear found it last year in South Africa It seems self-evident that Doctor Gear is helped by the knowledge that his African virus being a Type 4 is similar to those other Type 4 s that have been found in various places under similar or different circumstances

We have speculated at times about larger groupings of the viruse It seem to me that consideration might be given to a family of enteric viruses the *Enteroviraceae* with perhaps a tribe of *Parvovirae* We would favor such an identification rather than the scheme proposed by Mollaret but it raises the fundamental question of whether viruses should be classified in the same manner as bacteria While I do not subscribe to Mollaret s views which I fear are at best premature it would be equally justifiable to place the poliomyelitis viruses in a family of enteric viruses The poliomyelitis and Coxsackie viruses are so similar that I am frequently reminded of Jacob Henle s observation that like agents cause like disea es It may well be that more significant resemblances between the two are yet to be found

Virales

Family ENTEROVIRACEAE Members commonly found in the feces of infected pe sons and healthy carrie s

Tribe PARVOVIREAE Of small size eithe esist nt re tant to physical and chemical agents

Genus *Coxsackie* Un fo mly path genic for immature mice and hamsters Subcl n al nfection may occur in olde mice Indefinitely stable at −60 C and in 50 per cent glycerol at 4 C Ether res tant Rema n acti e for 7 da s at room temperature at pH 4 0 to 8 0 and for 30 m nutes at 49 C when su pended in phys ological salt sol ution contain ng 10 per cent beef infus on broth

(1 *Group* 1

H sts Man *Experimentally* s kling m ce and *hamsters* Chick embryo (ce tain strains of irus)

Geographical d stribution World wide

Seasonal d stribution A d sease of ummer and early fall

Indu ed d sea e In man fever headache muscular pa n nuchal rigidity suggestive of or n conjunction with po iomyel tis or pharyngitis with minute herpetiform lesions

(herpangina) In suckling mice and hamsters myopathic paralysis and early death severe generalized hyalin necrosis of skeletal muscles creatinuria. Infectivity of muscle far greater than that of brain

Size Readily pass membranes with APD of 30 mμ

Types At least 10 serologically distinct types

(2) *Group B*

Hosts Man Experimentally, suckling mice and hamsters

Geographical distribution Probably world wide

Seasonal distribution A disease of summer and early fall

Induced disease In man often fever headache, acute severe pains in lower thorax or abdomen (epidemic pleurodynia or myalgia) In suckling mice tremors ataxia and paralysis The skeletal muscles commonly undergo focal to generalized hyalin necrosis the brain encephalomalacia, the fat pads an unique form of necrosis. Muscle and brain of approximately equal infectivity Pancreas reported to undergo acinar necrosis in both immature and mature mice.

Types At least 3 serologically distinct types

THE COXSACKIE GROUP OF VIRUSES*

By Joseph L. Melnick†
Yale University School of Medicine New Haven Connecticut

The Coxsackie or C viruses pose an unusual problem in that soon after their discovery it was recognized by both Sickles and Dalldorf[1] and by ourselves[2, 3] that these agents exist in multiple antigenic types the total number of which remains to be determined There are a number of laboratories in this country[4-9] and abroad[10-14] engaged in typing strains but there is no central clearing house for the recording and designation of new types nor is there uniformity as to the methods employed either for classification or for nomenclature ‡ Furthermore the issue has not yet been settled as to whether we are dealing with different immunological types of a single virus or actually with different viruses

Laboratories have exchanged strains particularly those strains belonging to types which have been reported in the literature The lag between discovery and identification of a new type and its reporting in the literature meant however, that care had to be exercised not to give different antigenic entities the same designation This could easily occur with a general numbering system (as Type 1 Type 2 *etc*) in which the same number could be assigned by different laboratories to two different virus types I am sure that this difficulty has led to confusion in the other direction in that there must now exist certain distinct types which are known by more than one name

To avoid confusion with systems of nomenclature in use by other laboratories we have adopted a system of classification which depends solely on antigenicity and a provisional system of nomenclature which depends upon the area from which the prototype strain was first isolated The antigenic basis of classification in virology is time honored and more stable than any other

Before continuing with our method of classification based on antigenicity alone I should like to comment briefly on the fact that some of the C viruses differ in their pathogenic capacities as well as antigenically This has been recognized by Gifford and Dalldorf[15] in their classification of the C viruses into two groups based chiefly on the histopathological features of disease induced by different strains in infant mice In this scheme strains causing only generalized myopathy are classified in Group A and those inducing focal myopathy together with lesions of the central nervous system and fat are included in Group B The results of other studies[16] however suggest that the strains known at present are not clearly divisible into these two categories Instead they exhibit a gradient of pathogenic activity ranging from the Conn 5 type which induces variable injury to muscle and severe lesions of the central nerv

* A d d by gra t f om th N t on l F d t f I fant l P ly
† Th th xp es his d btedn t h ll gu D Guillerm C t ras for as ta th p epa rat f th p pe
‡ I 1950 tt mpt f th Am an Soc ty f Bact l log t t l this p blem by tt p S b mm tt th C k V ru m t w h f l when th members f th comm ee Drs G lb t D lld f H ld R Co and J eph L M l k ld t ch una im g em t

ous system fat, liver and pancreas, to the Texas 1 type, which causes severe muscle and cardiac damage, but only rarely lesions of the central nervous system, viscera or fat Effects intermediate between these extremities are produced by other C viruses particularly the Ohio-1 and Nancy types In studies of the histologic changes induced by C viruses *in infant mice destructive* changes in skeletal muscle were detected following infection by every one of 120 strains representing 16 *antigenically different types* Certainly skeletal muscle is the tissue most consistently affected by these agents

The distribution *of lesions in mice has been found to be influenced* by many factors[16, 18, 20, 21, 22] including the type and dose of virus the route of inoculation, *the strain and age of mice* the administration of cortisone, and the time following inoculation when tissues are taken for histologic examination In *our opinion* these variables make the histopathologic classification suggested by Dalldorf difficult and less certain than one based exclusively on immunologic criteria

To return to the system of nomenclature in use in our laboratory we have provisionally used the name of the place where the virus was first demonstrated as the type designation even though we are well aware that many types have global distribution The fact that four of our prototype strains bear the name Texas namely Texas 1 Texas 12, Texas 13, and Texas 14 is witness to the fact that several antigenic types may be prevalent in one area Other prototype strains bear names such as Alaska 5 and Israel 7 indicating that these types have been encountered for the first time in specimens collected in these areas The number following the place name is the strain number from a particular area Thus three strains from Connecticut numbers 3 5 and 7 are alike antigenically As the first strain of this type isolated was Conn 5 this strain has been considered a prototype strain

Before we can have a satisfactory system of nomenclature we should know just what we are naming I shall, therefore consider briefly the methods used *for the immunological* classification of C viruses Convincing evidence for the existence of multiple distinct antigenic types has been obtained by means of cross *neutralization tests in mice* (TABLE 1)[1, 4] by *cross* complement fixation tests (FIGURE 1)[4, 17] by cross protection tests in infant mice born of immunized mothers* (TABLE 2)[8] *and by cross protection tests in* chimpanzees[3, 18] The results obtained by each of these different methods of identifying strains of C virus have been in close agreement[3]

Not infrequently two types of C virus have been isolated in the same mice (or even in the same mouse) from specimens *of sewage flies* or pooled human stools[19] One instance is on record[20] of two C virus types isolated from the feces of a single patient With isolates containing *multiple* C viruses neutralization cannot be demonstrated with any one specific type antiserum When

B c em cq e a t l es t n t infect by C ruses a ly in f f t i n t po *bl t* n te wborn m a d lat te t f immun ty by d rect h ll ge Howev by chall g g inf t m les th n 48 h rs f g d born f p usly c ted m th rs cros protect test b c rried o t I the c rse f th xperime ts, it w s dem trated th t as *long* ted m th la tat suffi t protect afford d by h rt (e day) rsi g per od t p ev t a mm f t litte f m be ng *infected* by a chall g i j cti of h m l go v rus The mm ty f rr d by th fost m th is type pec fi T g th r w th th t *fe of imm* ty th j a multa eou tra f of c mplem t fi g tibod es from mothe t young It n tew rthy that n the hum pop lat pas tra *f of n t* li g d omple- m t fi g t bod es from the moth r to her ff p ng also occurs

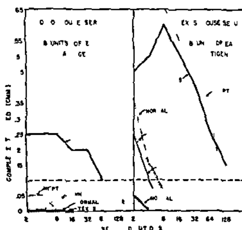
these isolates are investigated by means of the complement fixation test how ever reaction with different type specific antisera indicates the identity of the strains contained in the mixture and this may be confirmed by screening the

Table 1

Neutralization of C Viruses and Demonstration of Seven Antigenic Types

Type	Stra	Co t l ID	Imm Serum Log I t al zat Ind: C 5	Oh o-1	N ncy	T	H Pt	F	T T	Fleet	Ols
Conn 5	Conn 5	10⁻ ⁷	5 2	0	0	0	0	0	0	0	0
Ohio-1	Ohio-1	10⁻⁵ ²	0	4 7	0	0	0	0	0	0	0
Nancy	Nancy	10⁻ ⁹	0	0	5 5	0	0	0	0	0	0
Texas-1	Texas-1	10⁻⁷ ⁶	0	0	0	6 0	5 0	0	0	0	0
Texas 1	NHF 43	10⁻ ⁵	0	0	0	6 0	6 0	0	0	0	0
Te as-1	H gh Point	10⁻⁶	0	0	0	4 6	5 0	0	0	0	0
Dalldorf's 1	Easton 2	10⁻⁴	0	0	0	0	0	5 0	5 0	0	0
Dalldorf s 1	T T	10⁻⁶	0	0	0	0	0	5 0	5 0	0	0
Dalldorf s 2	Fleetwood	10⁻	0	0	0	0	0	0	0	6 0	0
Dalldorf s 3	Olson	10⁻⁶	0	0	0	0	0	0	0	0	4 5

Stra T s-1 NHF-43 and H gh P t b long t T xas-1 type St East 2 nd T T re t in ident l f h on d re l 5 d t x h D ll d rf Type I In s m la fash on in dd t on l types ha been d ot fi d Easton 10 East n-14 Ala k 5 I ra 17 T as 12 T xa 13 T 14 T 15 Bost

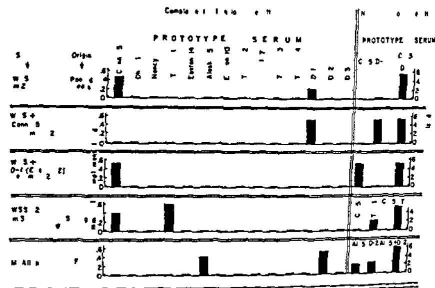


Fi ure 1 Examples of serological pec fic ty f two C iru types as d term ed by complement fixati tests R l t h p f mount f ompl m t fi ed with ry g d l ns f Oh o-1 nd T xas-1 immun serum the p f 8 units f Co 5 Oh o-1 T xas 1 d H gh P t ant g ns N rm l t t ge t d l t on f 1 2 and 1 32 also i luded

mixture through each of these sera to yield a single pure strain (figure 2) Consequently when antisera against strains of these types are used together complete neutralization of the mixture can be achieved This illustrates the value of utilizing both neutralization and complement fixation techniques for

TABLE 2
MATERNAL TRANSFER OF HOMOTYPIC IMMUNITY

Challenge V rus		B b es B rn of M thers Va cinated w th		
		C nn 5	Ohio-1	Te 1
Type	Stra n	Rat f su cept ble m ce to number oc 1 t d		
Conn 5	Conn 5	0/36		27/29
	N C		16/16	52/52
	NL-JM	1/27	13/13	58/62
Ohio-1	Ohio-1	17/17	3/172	46/46
Nancy	Nancy			27/27
Texas-1	Texas-1	42/42	41/41	0/47
	H gh Po nt	65/65	8/8	8/74
	NHF-43			0/25
Dalldorf's 1	Easton 2	46/50	67/6	62/6?



OCCURRENCE OF MULT PLE TYPES IN A S NGLE SOLATE

FIGURE 2 *L 1* The orig l W S strain h d lts o g n in pooled tools of pat ts w th paraly c poliomye l tis A c f t gen w prepa d from mic infect d with th rus ts econd mous p ss ge (indi ed m This nt g n ea ted i the f test w th Con -5 and *D lld rf Type* 1 er separat ly In tra zat on tests th viru was n tr l ed by n ther s rum l e b t o ly by a m e of b th

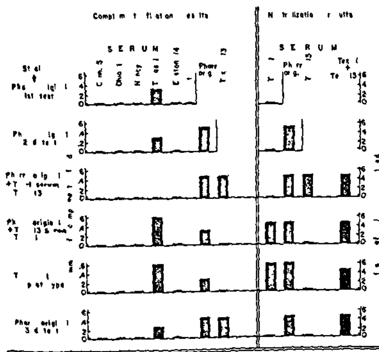
L ne 2 Aft r tw se l pa sages f the o ig n l rus in the p esenc f Con 5 immun serum th rus rea ted only with D lldorf s Type 1 i the f d in the neutr lizat test

L ne 3 After two s l pa ges of th or g al rus in th pres e f Eas 2 immun serum (Dalld rf Type 1) the virus reacted ly w th C nn -5 rum in th c f nd in th n tral zation tests

Line 4 In m r fash o th WSS 2 strai rea ted in th c f t t w th Co 5 d T 1 ra Al hough to small d gree t eacted n th n tralizat o test w th T xa 1 serum but t w th Con 5 rum t w comp t ly utral zed by a mixture f Co n 5 plus T x s-1 se

L ne 5 In m l r f hion the McAllen tr in wh ch w i lated f m fl es was f d to ta n tw type Al ka 5 and D lldorf s Type 2

the identification of new strains of C virus Had we resorted to neutralization tests alon we would have found that serum prepared against an isolate con taining two different type strains would neutralize the new isolate wherea no



SEPARATION OF TWO COMPONENTS FROM A SINGLE ISOLATE

FIGURE 3 O f the types separated from th gl isolat (Pharr 8) w f und t b ew t genic type

Line 1 Th orig nal tra was tested w th th 12 type known at th t m I th f test t re t d w th Texas-1 se um b t th rum f l d to ral ze t

L 2 S rum wh h was p p d ag t Pharr tra eact d w th th ig f Ph t a in both th c f d t l zat on test

Line 3 Th Ph rr tra was taken th gh tw pas ges in m ce i th p esen f T s-1 immun rum. It lo g rea ted w th Texa 1 rum in b f test. Immun rum w p p ed t th pas d rus and this ru w ll th rigi l Ph rr serum r ted in b th th f d ral za on tests Th serum failed to re t w th y f th p eviously known 12 types f rus th r test, d this rus and ts serum w cons dered th prot type tr in d s um f w type T as 13

L 4 In simil fash wh th or gi al Pharr virus wa pa d in th pres n f T 13 rum h rus recovered w found be singl type T as-1

Line 5 Th or gi l T xas-1 p type s ra f th type b h ed denti lly to on of th tra i lated f om th rig al Ph rr i la

L 6 Th or g l Ph rr tra n w s prov d to be mixt f T 1 d T xa 13 f t reacted w th ach of thes ra (as w ll as w h its h m logous serum) th f test b t ly w th m t f th tw prot type (ts h m l go rum) th neutral zat est

previously known specific type serum would do so This would lead to the erroneous establishment of a new type (really a mixture of two known types) which would show a one way cross with each of the known types in which the new serum would neutralize the two previously known types Even when we have resorted to the use of both complement fixation and neutralization tests

difficulties have arisen particularly (as shown in FIGURE 3) when the new isolate contained one known and one as yet unidentified type[19]

To summarize the results of our typing program I should like to present some data from our laboratory on the distribution of different C virus types according to the area and year where and when the specimen was collected (TABLE 3) Some strains were isolated from samples collected several years before the discovery of the C viruses these specimens having been stored in the frozen state The C viruses are global in distribution although all types have not penetrated to all parts of the world[22] Some outbreaks have been characterized by the isolation of only one type (as in Charleston, West Virginia, in 1951) others by the isolation of one predominant type (as in Easton Pennsylvania, in 1949),[23] and still other outbreaks in which 13 distinct types were recovered (as in Texas in 1948)

Table 3

Distribution of Antigenically Distinct C Viruses in Material Collected in Different Areas from 1942-1951

Area	Year	No. of Isolations	Antigenic Type															
			Conn 5	Ohio-1	Nancy	Texas 1	Easton 14	Alaska 5	Easton 10	Texas-12	Isra 17	Texas-13	Texas 14	Texas 15	Easton 2 (D 1)	Flood (D-2)	Ohio (D 3)
Texas	1942	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Conn	1943	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Texas	1948	34	3	—	4	7	6	2	3	5	1	1	2	1	1	2	12
Easton Pa	1949	28	—	—	1	—	1	1	1	—	—	—	—	—	24	—	—
W Va	1951	8	—	—	—	—	—	—	—	—	—	—	—	—	—	8	—
Others	1947-50	77	29	4	3	6	7	3	4	5	14	1	1	0	8	3	0
Totals		149	32	4	8	14	14	6	8	10	15	2	4	1	33	13	12

It would be helpful if we knew more about the distribution of C viruses in relation to human disease for it is possible that a classification could be devised along these lines Even though this kind of information is still meager and fragmentary it has been reviewed briefly[24]

Strains of the Connecticut 5 type were first recovered from one boy with pleurodynia and from five representative patients with pleocytosis of the cerebrospinal fluid who were acutely ill but not paralyzed during an unusual prevalence of aseptic meningitis in the summer of 1948 in Connecticut and Rhode Island[4] During the same summer, in various parts of New York state antigenically related strains were recovered from eight patients who likewise had acute nonparalytic illnesses associated with pleocytosis of the cerebrospinal fluid[26] At the same time a virus of this type was apparently responsible for a small outbreak in New York City of acute illnesses some of which resembled pleurodynia[23] A Conn 5 type of C virus also appeared to be the etiologic agent in an outbreak of pleurodynia which occurred during the summer of 1947 in Massachusetts[25] Thus, this single type of virus was apparently prev

alent during the summers of 1947 and 1948 in New England and New York and the cause of infection in cases of aseptic meningitis or pleurodynia

In these early studies relatively few attempts were made to recover the agent from patients with other forms of illness or from healthy individuals It is noteworthy that strains of this type were not encountered among a large number of specimens tested in Albany New York during 1949 [5] or in Washington D C during 1949 and 1950 [9] It was found in only one case in New Haven during 1949 and in none during 1950 and 1951 An Ohio-1 type was found together with poliomyelitis virus in representative patients during an outbreak of *summer grippe which occurred in 1947 in Ohio* [29] *Additional* strains of this type have not been encountered since During 1949 and 1950 in the vicinity of Washington D C six other antigenically different C viruses were isolated from patients with herpangina which appear to be capable of causing that syndrome [8, 9] At least two of the latter types (Dalldorf s Type 2 and our Texas 1) have also been recovered from patients with either poliomyelitis or minor illnesses without distinctive features A relation of other individual types of C virus to particular forms of disease has not been clearly established although certain types have been associated with patients simultaneously infected with poliomyelitis virus [23]

In conclusion we feel that C viruses can be clearly classified on an immunologic basis Over 160 strains have been classified in our laboratory and these have fallen into 15 antigenically distinct types Care must be exercised to exclude new isolates containing more than one type of virus from being set up as new prototype strains Further attention should be given to the possible relationship of human disease to certain C virus types A uniform nomenclature for the designation of these types is desirable and we hope that it will be soon forthcoming perhaps as a result of the joint efforts of certain members of this Conference

Addendum

Since this paper was presented several reports have appeared that bear on the characterization and classification of Coxsackie viruses One hundred and six strains isolated in Huebner s laboratory in 1949 and 1950 were found to fall into seven antigenically distinct types [33] The relationships of these types to the prototype strains used in Dalldorf s laboratory [4, 14] and in our own is shown in TABLE 4

It would be desirable to learn more of the biological properties *of each viral* type Only certain ones appear to multiply in embryonated eggs [34, 35, 36] and two strains have been found to produce an extensive destruction of striated muscle in the chick embryo [37, 35] Special strains have been found to multiply in cultures of mouse embryonic tissue [38] chick embryonic tissue [35] human embryonic and mature tissues [39, 40] and immature monkey testis [41] Actually a few strains have been isolated in human and monkey tissue cultures from stools that gave negative tests for C virus in newborn mice It was only after passage in tissue culture that these strains became pathogenic for mice [40, 4]

Concerning other properties of these viruses there are suggestions that all

prototype strains may not be of the same size (even though all that have been studied appear to be of the same order of magnitude)[46, 47, 48, 49] and that one prototype strain (Ohio-2) may be ether susceptible[50]

The difficulties of a classification based on histopathology have been emphasized in the recent demonstrations by Pappenheimer and Dalldorf and their associates[30, 42] of two lines of the Conn 5 strain with different tissue tropisms After serial passages of the strain in Boston (or New Haven) the virus produced pancreatitis in infant and adult mice, whereas in Albany the virus failed to produce pancreatic lesions Dalldorf and Gifford suggest that the serial brain to-brain passage of the strain carried out in Albany may account for the loss in the pathogenicity of the virus for the pancreas

Even though there are certain marked differences in the properties of the prototype strains, they appear to have certain antigenic components in common Thus, they exhibit heterotypic responses in the complement fixation

TABLE 4

*CROSSING OF PROTOTYPE STRAINS IN USE IN THREE LABORATORIES**

NEW HAVEN	ALBANY	WASHINGTON	NEW HAVEN	ALBANY	WASHINGTON
Conn 5	Group B Type 1		Easton 2 (Type 1)	Group A Type 1	Type 1
Ohio-1	Group B Type 2		Easton 10	Group A Type 8	H2
Nancy	Group B Type 3		Easton 14	Group A, Type 5	H1
Texas-1	Group A Type 4	Type 4	Type 2	Group A Type 2	Type 2
Texas-12			Type 3	Group A, Type 3	
Texas-13	Group B Type 4		Alaska 5	Group A Type 10	H3
Texas-14			Israel 7	Group A, Type 6	H4
Texas-15	Group A Type 7		Boston	Group A, Type 9	

* From Contreras *et al* [15]

reaction carried out with chimpanzee and human sera[43, 44, 45] (but not with mouse immune sera) This heterotypic response is so broad that patients or chimpanzees infected with a so-called Group A *or* a Group B strain show complement fixing antibody responses to strains belonging to both groups As far as has been determined similar heterotypic Coxsackie antibody responses have not been observed in patients or chimpanzees with non Coxsackie infections

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CLASSIFICATION AND NOMENCLATURE OF THE POLIOMYELITIS GROUP OF VIRUSES*

By Andrew J Rhodes

Connaught Medical Research Laboratories University of Toronto Toronto Canada

The task of the taxonomist seeking to classify the viruses pathogenic to man and animals is rendered difficult by the fundamental biologic nature of these agents which are essentially intracellular parasites Since they have, in general no colonial metabolic, fermentative or enzymatic activities that can be readily detected *in vitro*, it is not possible to make use of many of the properties which are so valuable in classifying bacteria Nevertheless, the task is not impossible if the taxonomist adopts suitable criteria for characterizing the members of this group criteria that take into account the intimate relationship existing between host and parasite Thus, the animal viruses can be arranged into families and genera, in the taxonomic sense on the basis of the nature and distribution of the pathologic changes in the host and epidemiologic features Further arrangement into species and subspecies can then be made according to the biologic properties of the individual virus agents

The classification of the viruses introduced by Holmes (1948) is based on such general principles This painstaking attempt to introduce an orderly arrangement of the viruses, parallel to that developed over the years for protozoa plants and animals, has met with a substantial volume of support from those best acquainted with the problems and techniques of taxonomy It has received a smaller measure of support from those less familiar with the highly specialized field of taxonomy

It is the object of this paper to present, against a background of present knowledge of this particular group of agents, a constructive criticism of the classification of the poliomyelitis group of viruses introduced by Holmes according to internationally accepted procedure (Buchanan, St John Brooks and Breed 1948) for it is my firm belief that this is the correct approach to the problem of arriving at an acceptable classification

First I shall present very briefly opinions regarding the classification of the poliomyelitis group that have been advanced by a number of other workers both before and after the publication of Holmes

The Poliomyelitis Group of Viruses

During the past few years the practice has developed of referring to certain agents as members of 'the poliomyelitis group of viruses' The viruses that have been placed in this group by various workers are the virus of human poliomyelitis the viruses of Theiler s encephalomyelitis of mice and the Teschen disease of swine and the Columbia SK and MM viruses of Jungeblut

Discussing first the virus of human poliomyelitis the biologic properties of this agent have recently been defined by the Committee on Nomenclature of

* In the preparat n of th paper I h v h d co de bl as ist nc from my colle gue D C. E an Rooy who is now ng g d on a c mpreh s re i n f D H lmes cla ifi t on

the National Foundation for Infantile Paralysis (1948) and by Paul (1949) These workers have expressed the opinion that the term poliomyelitis virus should be used to designate strains of the causal agent of human poliomyelitis and they have suggested that the chief biologic properties of this virus are as follows (1) Ability to produce lesions of characteristic nature and distribution in the central nervous system of man (2) Infectivity for primates who develop characteristic clinical and pathologic appearances (3) One group of strains known as the Lansing is transmissible to mice (4) Small size of the virus particles (5) Resistance of the virus to ether

More recently information of considerable value in the classification of poliomyelitis virus has been furnished by the Committee on Typing of the National Foundation for Infantile Paralysis (1951) This work which follows up earlier studies of Kessel and Pait and Bodian Howe and Morgan demonstrates that poliomyelitis virus as just defined exists in the form of three antigenic varieties or types known provisionally as the Brunhilde (1) Lansing (2) and Leon (3) It is of course well known that the members of the Lansing group are pathogenic to mice as well as primates (Armstrong 1939 Bodian 1949) and that apparently the Leon strain can also be adapted to rodents (Li and Habel 1951) It may be said therefore that the three strains differ from one another not only in antigenic structure but in other properties as well

The second virus that has been placed in the poliomyelitis group is that isolated by Theiler from mice There are many similarities between the biologic properties of this virus and human poliomyelitis and few would deny that in any formal classification the two agents should be placed in proximity (Theiler 1941 Gard 1943 Burnet 1945 van Rooyen and Rhodes 1948 Jungeblut 1951)

The suggestion has also been made that the virus of Teschen disease of swine should be closely associated with the above two viruses as a third member of the poliomyelitis group (Gard 1943) Although some may feel that the causal agent of the swine disease has been inadequately studied this suggestion would also appear to be reasonable

More controversial is the taxonomic relationship between the virus of human poliomyelitis and the members of the Columbia SK group Jungeblut (1951) has suggested that these viruses be placed in a group designated as parapoliomyelitis virus along with the human poliomyelitis viruses of the Lansing or rodent pathogenic variety This parapoliomyelitis group is closely associated in Jungeblut s classification with human poliomyelitis virus on the one hand and the viruses of Theiler Teschen disease and Coxsackie infection on the other I for my part do not think that the biologic and immunologic characteristics of the Columbia SK group warrant placement in such close proximity to the viruses of human poliomyelitis Theiler s disease or Coxsackie infection

Classification of Holmes

The above mentioned authors have not attempted a formal classification or adopted the binomial system of nomenclature and the only such scheme in

existence is that of Holmes (1948) In this classification the poliomyelitis group is placed in the family *Erronaceae* with the generic term of *Legio*, as shown in TABLE 1

As defined by Holmes the type species of the genus, *L debilitans*, comprises the human poliomyelitis virus and the Columbia SK MM viruses It will be evident that the genus *Legio* in addition to the virus of human poliomyelitis,

TABLE 1
CLASSIFICATION OF POLIOMYELITIS VIRUS (HOLMES 1948)

Order	VIRALES Breed Murray and Hitchens
Suborder	III *Zoophagineae subord nov*
Family	III *Erronaceae fam nov* Viruses of the Encephalitis Group inducing diseases mainly characterized by effects on nervous tissue
Genus	II *Legio gen nov* Viruses of the Poliomyelitis Group often recoverable from feces of the *infected hosts probably because of involvement of some part of the alimentary tract* usually there is also obvious involvement of some part of the nervous system Generic name from Latin *Legio* an army or legion The type species is *Legio debilitans spec nov*
Species	I *Legio debilitans spec nov* (poliomyelitis Col SK) II *Legio erebea spec nov* (LCM) III *Legio simulans spec nov* (pseudo LCM) IV *Legio muris spec nov* (Theiler) V *Legio gallinae spec nov* (avian encephalomyelitis) VI *Legio suariorum spec no* (swineherd s disease)

TABLE 2
GENUS *Legio* HOLMES PROPOSED AMENDMENT

Genus	*Legio*
Description	Viruses causing poliomyelitis in man recoverable from central nervous system feces and throat secretions producing lesions of characteristic nature and distribution in central nervous system of man and primates some strains also transmissible to rodents Generic name from Latin *legio* an army or legion The type species is *Legio debilitans*
Species*	I *Legio debilitans* The type subspecies is *L debilitans subsp Brunhilde*
Subspecies	I *Legio debilitans subsp Brunhilde* II *Legio debilitans subsp Lansing* III *Legio debilitans subsp Leon*

* Columbia-SK virus originally included in Species I *L gio debilita* , l t be pla ed in a ew fam ly together with African and Ameri an mosq to-born viruses (Smithburn Roc Garcia Hammo)

contains four species not seriously considered previou ly as having much in common with this virus (LCM, pseudo LCM avian encephalomyelitis and swineherd's disease) as well as the Columbia SK and Theiler's virus

I suggest that the biologic properties of the virus of human poliomyelitis first isolated by Landsteiner and Popper (1908, 1909) are sufficiently distinctive to justify placing this agent in a genus by itself I propose accordingly as shown in TABLE 2 that genus *Legio* Holmes be amended so as to include a single species only, the cause of human poliomyelitis

A full description of this genus would include reference to additional characteristics such as size resistance to ether growth in tissue culture and immunologic properties On the basis of the study of the Committee on Typing just mentioned I propose that the species be divided into three subspecies named *for the Brunhilde Lansing and Leon strains which differ one from another* in immunologic as well as biologic properties and of which full descriptions are available (Am J Hyg 1951 54 195–196) It is believed that the ranking of these strains as subspecies will be helpful in the future For example this arrangement will allow the listing as individuals of the remainder of the 100 strains typed by the Committee as well as allow for a possible later arrangement of these into types and groups Another development that can easily be taken care of is the creation of new species for poliomyelitis viruses growing only in tissue culture or for viruses of antigenic composition different from the Brunhilde Lansing or Leon strains

The type species of genus *Legio* becomes *Legio debilitans* The type subspecies of the species *Legio debilitans* becomes *Legio debilitans* subsp *Brunhilde*

Some five species in genus *Legio* Holmes remain to be considered and the

TABLE 3
PROPOSALS FOR CLASSIFICATION OF SPECIES II TO VI IN GENUS *Legio* HOLMES

Species	II	*L. erebea*	Constitute a new genus in family *Erronaceae* to include
	III	*L simulans*	these as separate species
	IV	*L m is*	Constitute a new genus in family *Erronaceae* to include Theiler s virus (3 Types) and the virus of Teschen disease as species
	V	*L. gall nae*	Place in genus *Erro* (fam ly *Erronacea*)
	VI	*L sua orum*	Reject evidence for viral nature inconclusive

proposals are shown in TABLE 3 In a detailed presentation of this proposal it will of course be necessary to publish descriptions of the new genera

Conclusions

In this brief and necessarily incompletely documented communication I have considered the problems involved in classifying the poliomyelitis group of viruses The biologic properties of this group of viruses are such that formal classification presents no particular problem I propose that the classification introduced by Holmes in 1948 be amended so as to take advantage of the newer knowledge of the properties of the individual members of this group Accordingly I propose that genus *Legio* Holmes now include only a single species *Legio debilitans* the cause of human poliomyelitis I also propose that the species be divided into three subspecies named for the Brunhilde Lansing and Leon strains The other viruses classified by Holmes in genus *Legio* should be dealt with by placement in new or existing genera or by rejection

In the amended classification use has been made of pathologic and epidemiologic features host range at the level of the genus and biologic and immunologic characteristics at the level of the species and subspecies

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DISCUSSION OF CLASSIFICATION AND NOMENCLATURE OF THE POLIOMYELITIS VIRUS GROUP

By Hilary Koprowski

Section of Viral and Rickettsial Research Lederle Laboratories Division American Cyanamid Company Pearl River N Y

My task in discussing the poliomyelitis virus group was made slightly easier perhaps by the fact that in 1948 a Committee on Nomenclature of the National Foundation for Infantile Paralysis formed *ad hoc* by the First International Conference on Poliomyelitis published a Proposed Provisional Definition of Poliomyelitis Virus [1] The scope of this study was to define the limits of the use of the term poliomyelitis virus The committee did not regard their report as an attempt at classification of poliomyelitis virus but rather as an attempt to remove some of the existing confusion regarding restrictions applied to the term poliomyelitis virus The principles upon which the committee based their recommendations stressed the clinical signs host range experimental disease and histopathological findings more than the actual properties of the virus

Our knowledge concerning the latter was and is still very meager For purposes of classifying the poliomyelitis virus group however one has to consider first of all the properties of the causative agent itself rather than the reaction of the host to the infectious agent Thus in line with many of the other authors of this monograph and with the principles agreed upon by the Fifth International Congress of Microbiology [2] I should like to consider the poliomyelitis virus group from the point of view of (a) morphology (b) susceptibility to physical and chemical agents and (c) immunological properties Following the suggestion of Sir MacFarlane Burnet one who scans the characters of various clones of viruses is struck by the possible systematic relationship between the agents causing infantile paralysis of man Theiler s disease of mice and Teschen disease of pigs My discussion will include these groups although our knowledge concerning the last member Teschen disease is very limited indeed

Morphology In TABLE 1 are summarized data concerning the shape and size of human poliomyelitis and Theiler s viruses as determined by electron microscopy It may be observed that in the two papers quoted the shape of the poliomyelitis virus particle is agreed upon as being spherical and that the values obtained for its size are 25 mμ and 20 mμ respectively [3,4] The particle size[7] of mouse encephalomyelitis virus as well as the sedimentation constants[5,9] are in very close proximity to those of the Lansing strain[5,6] and to those of an untyped strain obtained from human cerebral nervous tissue Recently electron micrographs illustrating the Brunhilde and Leon strains of poliomyelitis have been published and the size of the Brunhilde particle has been determined to be 20–50 mμ (spherical)[10] and that of Leon to be 12–15 mμ (rod shaped) [11] although confirming evidence is required before these data can be definitely accepted While discussing electron microscopy of the human poliomyelitis virus one should also bear in mind the study of Rhian *et al* [12] who

observed characteristic particles in the size range from 6 to 30 mμ in diameter obtained from both poliomyelitis infected and normal central nervous tissue of cotton rats and mice. Kausche and Bender[4] and Leyon and Gard,[7] respectively, have presented evidence, however, which seems conclusive enough to consider that the particles observed by these investigators represented virus and not components of normal tissue.

TABLE 1
SIZE AND SEDIMENTATION CONSTANT OF POLIOMYELITIS AND MOUSE ENCEPHALOMYELITIS VIRUSES

Virus	Strain	Shape	Size mμ*	Sedimentation constant (S)†	References
Poliomyelitis	Lansing	Nearly spherical	12–34 (av 25)	—	Loring *et al.*[8]
	Lansing	Spherical	20	—	Kausche & Bender[4]
	Lansing	—	—	160	Loring *et al.*[8]
	?	—	—	150	Gard
Mouse encephalomyelitis	FA	Spherical	28	—	Leyon & Gard[7]
	FA	—	—	152	Gard[6]
	FA	—	—	160–165	Leyon[8]
	FA	—	—	160	Gard & Pederson[9]

* By electron microscopy
† By ultracentrifugation

TABLE 2
SMALLEST INFECTIOUS PARTICLE SIZE OF POLIOMYELITIS, MOUSE ENCEPHALOMYELITIS, AND TESCHEN DISEASE VIRUSES

Virus	Average pore diameter mμ		Approximate particle size mμ*	References
	Passage	Retention		
Poliomyelitis	40	25	8–12	Elford *et al.*[13]
	35	30	10–15	Theiler & Bauer[14]
	58	13	15	Levaditi *et al.*[15]
Mouse encephalomyelitis	35	27	9–13	Theiler & Gard[16]
Teschen disease	30	18	10–15	Horstmann[17]

* As determined by ultrafiltration

TABLE 2 summarizes data concerning the particle size of viruses as determined by ultrafiltration. It may again be observed that the approximate particle size was determined to be almost the same for the three virus types. The smaller values obtained by this method as compared with electron microscopy can be explained by the fact that the ultrafiltration technique determines the size of the smallest infectious particle only.

Susceptibility to Physical and Chemical Agents

The viruses of poliomyelitis, Theiler's disease, and Teschen disease are ether resistant.[18, 19, 20, 21] This characteristic alone makes it possible to separate

them as a group from the viral encephalitides and from lymphocytic choriomeningitis virus[20] the latter of which has been classified rather unhappily as the same genotype as poliomyelitis in Bergey's Manual. Another characteristic of the poliomyelitis and mouse encephalomyelitis viruses is the sensitivity of these agents to desiccation.[16, 22] Thus if the size of these three types of viruses is accepted to be 10–15 mμ or 20–28 mμ based on ultrafiltration and electron microscopy respectively the differentiation of the poliomyelitis group of viruses from the other ether resistant virus groups of similar size will be narrowed to the Coxsackie viruses and the Columbia SK Encephalomyocarditis group.

It may be observed in TABLE 3 that the viruses of Columbia SK-encephalomyocarditis poliomyelitis and mouse encephalomyelitis are very sensitive to desiccation procedures. According to Dalldorf[23] five preparations of Group A Type 1 Coxsackie virus lost two logs in titer following lyophilization. On the other hand lyophilization followed by benzene extraction (twice) ether extraction and rehydration in that order rendered the D2 and Texas strains of

TABLE 3
CHARACTERISTIC PROPERTIES OF ETHER RESISTANT GROUPS OF VIRUSES SIMILAR IN SIZE TO POLIOMYELITIS VIRUS

Virus	Sensitivity to desiccation	Hemagglutination of sheep RBC
Col SK-encephalomyocarditis	+	+
Coxsackie	± (?)	−
Poliomyelitis	+	−
Mouse encephalomyelitis	+	−
Teschen disease	Unknown	−

+ indicates that titers were markedly decreased.

Coxsackie virus still viable for mice.[24] Applied to a preparation of the MEF1 strain of poliomyelitis virus of comparable titer the same sequence of procedures completely destroyed its viability.

All members of the Columbia SK-encephalomyocarditis virus group agglutinate sheep red blood cells.[25] To my knowledge nobody as yet has been able to demonstrate specific agglutination of sheep RBC by the poliomyelitis,[25, 26] Theiler's[25] and Teschen viruses[17] although the GDVII strain of Theiler's virus was found to agglutinate human Type O cells.[27, 28] Of course one should bear in mind that future improvements over the present methods of desiccation may make it possible to dry the poliomyelitis viruses successfully and it is also possible that a specific hemagglutination test may be discovered tomorrow for the poliomyelitis Theiler and Teschen viruses. Specific conditions of the performance of the test[15] however may again help to distinguish additional properties of this group of viruses. It may also be worth while to notice that the various strains of Coxsackie virus may be found to differ in sensitivity to the desiccation procedure. In view of the extremely large membership of this particular virus group one should perhaps refrain from making generalizations based on only a few examples. Moreover the decrease in titer of the

poliomyelitis virus preparations immediately after desiccation was found to be three logs or greater and rarely was the virus viable after storage in the lyophilized state

Immunological Reactions

With the exception of influenza virus there are few, if any, viral agents that are better represented than human poliomyelitis virus in the variety of samples of different origin The admirably accomplished task of typing 100 of such strains resulted in grouping them in three immunologically distinct types Type 1 Brunhilde like, Type 2, Lansing like, and Type 3 Leon like[29] So far as we know there is no evidence which would deny the immunological distinctness of these three types in relation to each other and in relation to other viral agents *

Strains of Theiler's virus which apparently may be isolated from a mouse colony anywhere in the world, seem to be closely related serologically although they may quite often differ in virulence At least no evidence to the contrary has been furnished as yet Strains of Theiler's virus are immunologically unrelated to those of human poliomyelitis[11, 32, 33] to the Columbia SK encephalomyocarditis group,[34, 35] or to those of Teschen disease[17]

Although many strains of Teschen virus have been isolated in Europe and Africa[17] there is no evidence available, as yet that these strains are unrelated immunologically to each other

Besides these there are very few other characteristics that would be of help in planning a systematic approach to the possible classification of poliomyelitis viruses Although there are at least two papers dealing with the chemical composition of animal viruses, including poliomyelitis virus[36, 37] I believe the time is by no means ripe even to discuss this problem until more extensive evidence becomes available I shall refrain from discussing such criteria as natural methods of transmission and host, tissue and cell tropism because although attempts may be made to ascribe to the human poliomyelitis Theiler and Teschen viruses affinities for a particular host or tissue these are by no means characteristic I am not qualified to discuss histopathological lesions of the central nervous system induced by each of the above three viruses and in any case I seriously doubt the specificity of these lesions

Thus we arrive at the basic question Is our knowledge of the poliomyelitis group of viruses which is based on studies by electron microscopy ultracentrifugation ultrafiltration susceptibility to physical and chemical agents and immunological relationships, extensive enough to regard the poliomyelitis group as ready for taxonomic treatment? The answer obviously is 'No Many arguments can be furnished in support of this negative attitude For instance electron microscopy and ultracentrifugation studies dealt only with laboratory adapted strains and there is no guarantee that these did not represent mutants of the original wild strains Sensitivity of the poliomyelitis virus group to desiccation may have been conditioned solely, as mentioned above by the in

* The o l ex pt n t th s st tem t h s be n furn hed c tly by Ch ng a d Venn who t ed f lose serol g i elat hip betw n Type 2 Lan g l k tra f h m po omy l t s and th ee t g bovi e enceph lomy l t *Further work* w l d t th ow add t n l ght on th s int rest ng a d puz l g phenom n n

adequacies of the present methods and media of lyophilization The immunological distinctness of human poliomyelitis Theiler s virus and Teschen disease may have been due to the fact that owing to the limitations of our present methods and techniques only one antigenic complex of each particular virus group was studied Consequently surprises may still be awaiting us in the future Finally, the complete lack of knowledge concerning the evolutionary ' history of the poliomyelitis virus group furnishes one more argument which seemingly disqualifies this group of viruses as a candidate for classification Yet if the question were raised Is there a n ed to classify the poliomyelitis virus group? the answer would definitely be in the affirmative for the following reasons

To a large extent classifications are created for the convenience of the investigator when the need for a taxonomical order arises Such a need may be conditioned by several factors among which the confusion reigning in this particular field should be considered as of paramount importance More investigators seem to have been attracted to work in the field of the poliomyelitis virus group than in any other virus groups A few of these investigators including the authors of Bergey s Manual have conceived their own particular system of classification and have either created new states of confusion or added to the existing ones The need to dispel the present confusion is a strong argument in favor of discussion of a tentative classification and perhaps there is one further reason Pessimistically speaking I believe it unlikely that much more will be added to clarify the problem of classification and nomenclature of the poliomyelitis virus group if the current trend of investigations is continued Conversely if an attempt to classify these viruses will make manifest the meagerness of the criteria adopted for classification this in itself may serve as a stimulus to orient some research in directions that may prove to be more fruitful from a taxonomical point of view

Now turning to the actual classification if we adopt the working rule suggested by Dr Burnet that viruses falling in one genus have approximately similar size and appearance in electron micrographs and at least one functional characteristic then the viruses of human poliomyelitis and Theiler s disease may be considered as one genus As indicated above these pathogens have been determined to be of comparable size and shape by electron microscopy and ultrafiltration Moreover these two groups of viruses plus Teschen virus are ether resistant are sensitive to presently employed techniques of desiccation and were found not to agglutinate sheep erythrocytes The obviously weak points in this argument are those based on electron microscopy and following my previous statement this field of investigations should be highly encouraged with particular emphasis on the study of unadapted wild strains Recent studies of Enders *et al* [19] on direct tissue culture adaptation of naturally encountered strains of human poliomyelitis virus may certainly be considered as a source of suitable material for such experimental purposes The Teschen disease virus has not been as well investigated as the other two members and it may be provisionally included in the genus until further study particularly by electron microscopy ultimately establishes its position

Species within the genus will contain each of the three naturally occurring

groups human poliomyelitis, Theiler s disease, and tentatively Teschen disease Once this is agreed upon, the question of name is of secondary importance I am personally not so enthusiastic about the use of derogatory epithets as generic names as Holmes was I consider that the attachment to the genus of the name of an investigator who made outstanding contributions in the field is a much better practice Thus if we consider the discovery of a susceptible experimental animal in the monkey as the outstanding contribution[39, 40] (and our verdict will probably be unanimous), a name associated with Karl Landsteiner should be considered for the genus although such a name as *Landsteineriola* has been called "appallingly inconvenient' * As seen in TABLE 4 the three species would then be named *L levaditii*, in honor of the investigator who first proved the filterability and the *eo ipso* virus nature of human poliomyelitis virus;[41] *L theileri*, a name which does not need justification, and tentatively, *L kloboukı*, for the discoverer of Teschen disease I would not go beyond the speciation and would provisionally list the three so-called types

TABLE 4
PROPOSED NOMENCLATURE FOR THE POLIOMYELITIS VIRUS GROUP

Genus	*Landsteineriola*
Species	*Landsteineriola levaditii* var Brunhilde var Lansing var Leon (Viruses of human poliomyelitis)
	Landsteineriola theileri (Viruses of mouse encephalomyelitis or Theiler s disease)
	Landsteineriola kloboukı (tentative) (Viruses of Teschen disease)

of human poliomyelitis virus as varieties The question of definition of the species would of course, be raised and perhaps the following may be considered in answer *L levaditii* is the agent responsible for epidemics of human poliomyelitis, including the one in Baltimore in 1939, from which the parent strain variety Brunhilde was isolated the one in Lansing in 1937, from which the variety Lansing was isolated and the one in Los Angeles in 1937, from which the variety Leon was isolated These type strains are all deposited in the Viral and Rickettsial Registry U S A Any strain immunologically identical with any one of the above type strains will be included in the species *L levaditii* Similar definitions may be coined for *L theileri*, and later for *L kloboukı*

In offering the proposed classification I should like to avoid all questions of priority since only thorough consideration and discussion of the data available for taxonomic use can eventually give rise to a rational and useful scheme of nomenclature I am well aware that these proposals should be considered *only* as an account of the difficulties I have encountered the weaknesses I have had to try to overcome and the mistakes into which I have *fallen*

* I d ise tho fo whom th nam *Land i ersol* la ks eupho y, *to cons* d r th am *Es herichia* and t explain why the latte sound t ral and the f rmer d culous I is strongly s ggest compa i g the mo u m ntal w k of Land te e w th th t of E ch h i th fi ld f med l and b logical rch

Since I presume that many virus research workers feel similarly unqualified as taxonomists I should like to close by describing an attempt at classification in an entirely different field by a man as unqualified as many of us are but whose labors met with success All of you who are familiar with the music of Wolfgang Amadeus Mozart have probably noticed that his compositions do not bear the customarily used Opus number Instead Mozart s sonatas symphonies concerti and opera (his entire phenomenal output) are numbered in a single sequence and the number of each composition is preceded by the capital letter K K stands for Ludvig von Kochel who classified the works of Mozart Was Kochel a musician? Not at all Kochel was a botanist a taxonomist whose only qualification for his task was familiarity with the principles of scientific classification and love for Mozart s music Köchel s system is by no means perfect but thanks to him all of Mozart s compositions have been unearthed and published and this system is now universally used as the means of identification of Mozart in concert programs and in all writings upon the works of the composer Let this example be a consolation to all of us Love s labor is not lost

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VIRUSES OF THE ENCEPHALOMYOCARDITIS GROUP

By Joel Warren

Department of Bacteriology Army Medical Service Graduate School Washington D C

The viruses commonly designated as Columbia SK MM encephalomyocarditis and Mengo encephalomyelitis (which I shall call the EMC Group") comprise a family of strains the confused history of which affords an example of the needs for rigid and uniformly acceptable standards of identification and criteria of individuality They also provide a group readily amenable to systematization

In fact there are no specific characteristics within this generic group and the designation of these viruses as separate strains rests in the final analysis on diversity of origin only The worker given four unlabeled containers of each of these viruses would be unable to differentiate and tag them using any of the techniques now at our disposal On the other hand, he would have little difficulty distinguishing the family from all other known genera of filtrable agents by the criteria of tissue tropism and serological character Therefore I do not propose to elaborate on the details of the various strains but rather to define the general properties of the group and try to assess the differential value of their characteristics

The first of these agents to be isolated was recovered from wild cotton rats which were being used for the passage of the Yale SK strain of poliomyelitis virus The new agent differed antigenically and in its pathogenicity from the Yale SK and was designated as the Columbia SK virus A similar strain MM was isolated shortly thereafter from hamster brain originally inoculated with human spinal cord and medulla from a fatal case of an undiagnosed paralytic disease Because both strains were originally discovered under circumstances where either known or suspect poliomyelitis-infected material was being passaged through rodents considerable confusion surrounded the relationship of those strains to poliomyelitis virus and it was thought that they represented murine variants of the latter Subsequent study has failed to provide evidence of any common antigen between the agents of the encephalomyocarditis group and poliomyelitis and although similar in most physical properties they appear to be unrelated viruses

The encephalomyocarditis (EMC) and Mengo encephalomyelitis viruses were both originally isolated from captive monkeys the former in Florida and the Mengo virus in Uganda Subsequently a strain of Mengo virus was obtained from mosquitoes and a wild mongoose caught in the vicinity of the monkey compound at Entebbe Uganda

This wide geographic distribution would implicate a common generic host, and the pathogenesis of EMC viruses in the rat would suggest certain rodents as the natural reservoir The occurrence of protective antibodies in wild rats of certain geographic areas and not in others lends considerable support to this theory In the case of one strain Mengo arthropod carriers has been repeatedly demonstrated and these may well serve as vectors ... an reg...

Human infections with the EMC and Mengo strains have occurred and, in all instances, close proximity to rodents has been noted

All members of the EMC group possess the same antigenic composition, and are indistinguishable by standard immunologic procedures Variations between strains include *infective titer*, *invasiveness* by specific routes and greater infectivity for certain hosts None of these variations however affords a basis for strain differentiation, since any one strain manifests considerable lability in these respects, and since such variations are readily produced or modified with a given strain of virus by suitable passage and adaptation For example, EMC after repeated intraperitoneal passage in mice loses much of its neurotropism, and reaches high concentrations in the viscera The process is readily reversible by again passing the strain *via* the C N S

The following information might serve as the basis for a scheme for the systematic classification of the agents in the EMC group

Genus Name to be determined

Species Names to be determined (common names Columbia SK virus, MM virus, Encephalomyocarditis virus, Mengo encephalomyelitis virus *etc*)

Biochemical Properties The virus is a spherical particle, 25 to 30 mμ in diameter having a sedimentation constant of approximately $150 \cdot 10^{-13}$ It retains its infectivity for long periods at sub zero temperatures in 50 per cent glycerine or in 0 05 molar glycine, but not in the lyophilized state Its thermal inactivation temperature is 60°C for 30 minutes It is absorbed to, and agglutinates sheep erythrocytes at 4°C at an optimal NaCl concentration of 0 075 molar

Hosts Isolated in nature from cotton rat, hamster, mongoose rhesus monkey, chimpanzee and mosquitoes of the genus *Taeniorhynchus* Specific antibodies found in several species of wild rats, monkey and man Experimental infection in mouse rat hamster cotton rat guinea pig rabbit, rhesus monkey and embryonated egg

Experimental Disease (Host, mouse strain, mouse adapted)

Minimal incubation period, 18 hours after intracerebral inoculation

Susceptible routes all common routes

Infectivity range Intracerebral LD 50 per cent is 10^{-7} to $10^{-9.5}$
Intraperitoneal LD 50 per cent is 10^{-8} to $10^{-9.0}$

Clinical a rapidly progressing polioencephalitis with animals showing ruffled coat and paralysis but usually without coarse tremors or conjunctival hemorrhage Animals of all ages are susceptible and die in coma without convulsions

Pathological the essential lesions are inflammation and necrosis of skeletal and cardiac muscle and a diffuse polioencephalomyelitis affecting the entire central nervous system

Other Host Responses of Differential Significance Guinea pigs and monkeys run a variable course from paralytic fatal disease to inapparent infection

Serological Differentiation All members of the genus cross react by cross-protection, neutralization, and complement fixation and hemagglutination inhibition tests The genus is not known to contain any antigen in common with other viruses

General Information It will be noted that the important differential generic characteristics that serve to distinguish this group from other known viruses are

(1) Small size and reactivity with the surface of the erythrocyte

(2) A very broad host range including perhaps arthropods

(3) High infectivity titer by peripheral routes in a wide variety of adult hosts and clinical disease in these hosts

Characteristics other than locale which could be employed for strain differentiation have not been encountered in our experience

A word is in order regarding the title encephalomyocarditis for the group This was selected as an interim descriptive designation at a time when the identity of that particular strain with the (previously isolated) Columbia SK and MM viruses or Mengo virus was not suspected It is admittedly a cumbersome term and one devoid of respectability on a priority basis On the other hand it appears more descriptive than any of the four and free of confusing associations with other virus agents In its favor let it be said that it will be readily expendable when an alternative title is selected

In conclusion I wish to point out the desirability of the acceptance of at least a tentative scheme of virus and rickettsial groupings without further delay It is believed that if we can establish genera and species on an accepted basis of characterization and differentiation the names by which these groups will be called will evolve *sui generis* out of the needs of workers and teachers for some convenient title by which to designate the groups While individual feelings concerning a nomenclature are understandable as Sir MacFarlane Burnet has stated Probably the outstanding feature of the evolutionary process in parasitic microorganisms is the unimportance of the individual I am sure this applies also to virologists in search of a nomenclature

THE VIRAL AND RICKETTSIAL REGISTRY, U S A

By Joseph E Smadel

Department of Virus and Rickettsial Diseases Army Medical Service Graduate School Washington D C

The Viral and Rickettsial Registry provides the only public animal virus distributing center now in existence This presentation will be concerned primarily with the organization and maintenance of the Registry and with its possible contributions to the virological field

Much of the travail which accompanied the birth of the Viral and Rickettsial Registry is neglected in the following statements which are taken from the Foreword of its Catalog published in 1950* ' The Viral and Rickettsial Registry was instituted to fill the growing need for an available stock of documented viral and rickettsial agents It was formed by a confederation of investigators in the field who agreed to contribute free of charge for one year the specimens needed to create a functioning distribution center The present list of viral and rickettsial agents includes those mo t commonly used in research and teaching Without the encouragement and support of the Society of American Bacteriologists and the Committee on American Type Culture Collection of the National Research Council and the willingness of the American Type Culture Collection to assume the burden of serving as a repository and distribution center the Registry would not have been possible The actual cost in labor and money of maintaining the viral and rickettsial strains of the Registry and of preparing the specimens for distribution cannot be estimated but it is safe to say that it would be beyond the capabilities and budget of any single organization The Registry, therefore can exist only so long as investigators in the different research laboratories are willing to contribute their knowledge effort and interest toward its advancement The participants in the Registry include not only those who are actually supplying specimens but also those who have helped in various ways The group is composed of representatives from most of the laboratories in the United States where work on viral and rickettsial agents is pursued

At the time of its formation the contributors to the Registry established certain guiding policies which are not mentioned above Some of these were (1) The appointment of the subcommittee for the selection of materials to approve and recommend the microbial agents to be included in the Registry (2) The distribution of the microbial agents in the lyophilized state whenever feasible or in other instances, in glycerin or in dry ice (3) The charge of a reasonably high fee for each specimen in order to provide funds for the maintenance of the collection and to discourage indiscriminate requests by persons with only ca ual interests (4) The entering in the Catalog of detailed information on the passage history of the agents and sufficient data on their pathogenicity in order to enable the neophyte to establish and maintain the strain

After the first year of operation the contributors decided to continue the

* The Catalog of the Viral and Rickettsial Registry is available on request from the American Type Culture Collection 2029 M Street N W Washington 6 D C

Registry and additional policies were evolved These included (1) The investigators who originally contributed samples of a given strain agreed to assume responsibility for reassaying annually any material remaining at the end of a year or to supply the distributing center with new material This was to be done without financial recompense (2) The investigators also agreed to be responsible for the maintenance of designated strains until such time as they notified the Registry to the contrary These strains were to be kept as close as possible to the passage initially placed in the Registry

Since the Registry operates on a collaborative basis and since virologists seem to be unusually individualistic people it is not surprising that there have

TABLE 1
VIRAL AND RICKETTSIAL AGENTS CURRENTLY AVAILABLE IN VIRAL AND RICKETTSIAL REGISTRY

	VIRUSES	
Colorado tick fever	Encephalitis	West Nile virus
Coxsacki	Japanese	Psittacosis (6 BC)
Connecticut 5	Russian	Lymphogranuloma venereum
High Point	St Louis	Ornithosis (P-4)
Nancy	Eastern equine	Meningopneumonitis
Ohio-1	Western equine	Mouse pneumonitis
Dengue	Venezuelan equine	Feline pneumonitis
Herpes simplex	Encephalomyocarditis	Vaccine virus
	Lymphocytic choriomeningitis	Mouse neurotropic
		Chorioallantoic
Pseudorabies	Mouse encephalomyelitis	
B virus	TO	
Influenza	FA	Yellow fever (17 D)
A (PR8)	Poliomyelitis	Fibroma
A prime (FM1)	Lansing	Myxoma
B (Lee)	Brunhilde	Fowlpox
C (1233)	Rabies	Laryngotracheitis
Swine	Street	Newcastle
Mumps	Fixed	Pneumonia virus of mice
	RICKETTSIAE	
Epidemic typhus	Rocky Mountain Spotted Fever	Q fever
Murine typhus	Rickettsialpox	Vole rickettsia

been divergences of opinion on certain points For example there is lack of unanimity as to whether the Registry should serve as a depository for any agent which a contributor may wish to provide or whether it should restrict its facilities to a group of agents of special importance in research and teaching Up to the present the majority continues to favor the latter course Another question which has repeatedly arisen in the discussions of the contributors centers around the feasibility of supplying standardized antisera against the agents in the collection It has been the consensus that this would be most desirable However no suitable means for obtaining such a supply of sera has been forthcoming and the contributors have not thought that they could assume this additional burden

The great majority of the contributors to the Registry have been vociferous in expressing their dislike of any of the available binomial systems of nomen

clature for viral agents, insisting that all strains be listed in the catalog under their common names The agents currently available in the Registry for distribution by the American Type Culture Collection are given in TABLE 1

During the first year of its operation, the American Type Culture Collection distributed 110 viral and rickettsial specimens from the Registry During the second year, the number was 149 Since its organization the ten most frequently requested agents have been herpes simplex, lymphocytic choriomeningitis influenza A poliomyelitis (Lansing) pneumonia virus of mice, murine typhus vole rickettsia mumps rickettsialpox, and western equine encephalomyelitis Up to the present only two agents listed in the Catalog have not been requested

The Registry was organized originally for the benefit of investigators and teachers in the United States There has been a small but increasing demand for specimens by virus workers outside of the United States These requests have caused some administrative troubles to the American Type Culture Collection in the form of monetary-exchange and transportation problems but these can be solved Until the requests from other countries become numerous the contributors will probably be willing to supply materials for distribution to their foreign colleagues If the overseas demand becomes excessive, the organization of one or more virus-distributing centers in other parts of the world will be warranted

One might reasonably ask whether the Viral and Rickettsial Registry has accomplished anything beyond the provision of a utilitarian exchange mart for virologists It is my opinion that even the process of developing the Registry had an appreciable effect on the thinking of the contributors It created an opportunity and a demand for re-examination of the histories of the viral and rickettsial agents that were in constant use It diffused knowledge about the need for maintaining the classical strains under stabilizing conditions which would minimize the rate of appearance of variants Furthermore, for the first time a number of the contributors were impressed with the desirability of maintaining and distributing a viral agent that was not far removed from its susceptible native host and which had undergone the minimal number of alterations usually accompanying a long series of animal passages One may anticipate an increasing application of these principles in future work

This has been a rambling historical account of an unique experiment in virology The story has been purposely abbreviated Perhaps certain of the lengthy and heated discussions among the contributors should have been presented in some detail instead of being summarized as one sentence statements of policy In my opinion however the important thing is not the difference in points of view but rather the fact that sufficient unanimity prevailed to arrive at workable policies and to establish a functioning organization

PLANT VIRUS TYPF CULTURE COLLECTIONS

By H H McKinney

Bureau of Plant Industry Soils and Agricultural Engineering Agricultu al Research Administ ation U S Department of Agriculture Beltsville Maryland

Any nomenclatural system mu t be based on types Only when it is im possible to preserve the types becau e of the nature of the material should a system be based entirely on descriptions drawings and photographs

Linnaeus and some of the earlier botani ts were aware of the difficulty of making a truly complete written description of all of the characters of a seed plant species They recognized that at least the floral and foliar organs of each species should be preserved in herbaria to be made available for study by many investigators over the years The importance of this system was so clearly evident that practically all taxonomists since Linnaeus s time have preserved their types when the nature of the material permitted

With the growth of the genetic concept and the resultant realization that the species is not fixed but composed of many genotypes there has developed a need for the preservation of the living germ plasm of numerous genetic types of many important species To this end plant breeders and geneticists pre serve many of their seed stocks I reservation has become so extensive and so important in the United States that plans are being made for the development of a suitable large scale storage facility to care for important seed stocks and to permit the periodic re culture of stocks before their viability is exhausted Mycologists algologists bacteriologists and protozoologists have long recog nized the need for maintaining important species and strains in culture as evidenced by the many culture collections in laboratories throughout the world

Although the type concept originated largely with considerations of the species it is evidently no less important for varieties and strains Furthermore the preservation of types is determined not only by taxonomic interests but by many other scientific and technical interests as well

Among the students of the plant viruses it is not uncommon to hear objec tions to the maintenance of type collection on the ground that many of the viruses are known to mutate and therefore will not remain stable Obviously this objection begs the question Mutation has become a recognized attribute of living matter and we must learn how to deal with it A practice of going back to nature for a virus each time we wish to study it will hinder progress in studies directed towards the resolution and proper understanding of those biological physical and chemical characteristics that remain sufficiently sta ble to provide a sound basis for classification

Methods for Preserving the Plant Viruses

Viruses that cannot be transmitted by manual methods of inoculation are maintained in the living plant whereas many of the viruses transmissible by manual methods may be carried for varying periods in the extracted plant juice and in the dead tissues The tobacco-mosaic virus most of its mutants and certain of its allied viruses can be preserved for many years in air-dried

tissue and usually in extracted plant juice, when stored at room temperatures A few other viruses can be preserved for a few months to a year in this manner, but most of the viruses that can be transmitted by manual methods of inoculation require special treatment for preservation Many of these relatively unstable viruses remain viable for several months or more in extracted plant juice stored in a frozen condition

Viruses should be carried in stored preparations whenever possible, as their continuous culture in living plants is not only costly but also increases the chances for mutation and for contaminations from outside sources Although old preparations sometimes reveal a virus that was not known to be present in the original colony it appears that these are only trace contaminants which possess relatively high survival characteristics Some mutants may be detected in this manner however In some cases the symptoms induced by an old preparation differ slightly from those induced by the original culture suggesting that the original culture was composed of two or more very similar strains some of which did not survive storage An understanding of this problem depends on the perfection of methods for the accurate identification of strains that differ only slightly and are essentially compatible in the plant

In appraising preservation methods it should be pointed out that most viruses stored in frozen plant juices or in frozen moist tissue lose their viability rather quickly if by accident, the preparations become thawed and subjected to laboratory temperatures On the other hand, properly dehydrated virus preparations tend to lose their viability slowly when subjected to laboratory temperatures

Working with the relatively unstable viruses of potato veinbanding mosaic and potato Canada streak Dykstra and Du Buy[2] found that these viruses remained highly active for at least four months when the plant juices were extracted in an atmosphere of CO , then lyophilized and stored in sealed glass tubes held at room temperature

Working with potato virus X Bercks[1] found that the viability was retained for at least a year in solutions containing from 40 to 60 per cent glycerine, when stored at 3 0 C or at room temperature Bercks s preliminary tests with the potato veinbanding mosaic virus were not conclusive

To a limited extent the author has explored the preserving possibilities of glycerine, toluene dioxane and medicinal mineral oil with a few viruses In these tests fresh virus infected leaves were used Tobacco leaves were cut in strips about two cm wide but grass leaves were intact The fresh tissue was placed in the chemical in stoppered bottles, and stored at temperatures slightly above freezing To prepare the tissues for assay, the excess glycerine was removed by rinsing the tissue in water the mineral oil was removed with toluene and the dioxane and the toluene were removed by evaporation

The results obtained with the glycerinated preparations are shown in TABLE 1 All of the preparations with the other chemicals were found to be inactive when the glycerinated preparations were tested It will be noted in the table that the tobacco ringspot virus and the turnip mosaic virus show less activity in the glycerinated preparations than they do in the preparations that were dehydrated by means of $CaCl_2$ and Anhydrone It will be observed also that

10 days exposure to laboratory temperatures reduced the viability of the potato veinbanding virus and depleted that of the tobacco ringspot virus Ten days are about the maximum tim for transport through the mails in the United States Thus, a virus preparation that does not remain viable for this length of time at laboratory temperatures will require dry ice packaging

The method of dehydration of plant viruse *in situ* by chemical desiccants was described briefly by the author[3, 4, 5] for several viruses that are quite unstable under ordinary conditions Some additional information has been collected since those reports The method now used is described here in some detail

TABLE 1
RESULTS OF THE PRESERVATION OF CERTAIN VIRUSE IN LEAF TISSUE

V r.	Prepara-t on o.	Preserva t		Act vity† in		D hydr t d ta d rds in toba co‡
		Cod t mp months	Lab temp d	t b cco	wh t	
Cucumber mosa	368	8	0	5/5	—	—
Potato ve nbanding mosaic	309	7	10	3/5	—	
Pota o veir band ng mosaic	369	8	0	5 5	—	—
Tobacco ringspot	372	6 5	10	0/5	—	—
Tobacco ringspot	37?	7 5	0	5/5	—	—
Tobacco ring pot	3 2	7 5	0	0 05 les per cm²	—	1 0 les per cm
Tu nip mosa c	36	8	0	1 3 les per cm²	—	5 4 les pe cm
Wheat yellow mosa c (soil borne type)	380		0	—	11/57	—

Leaf tiss was red glyce in t temperat lightly bov f a g f llow d by l bo tory t mperat res with tw f the preparation Gl cr in reage t grad was dil ted to 5 5 pe t w th di illed w t conta ning 8 5 grams of N Cl per l t r The tiss w k pt submerg d in the glycer ne by means of large rubber stoppers

† Th ume tors of th f action den t th umbe f plant how ing fect on d th d nomina or d not th umbe f plants inoc lated Th h d ta the column d ot th numbe of local necrotic les ons pe sq cm of leaf fac w ped w th th p paratio

‡ Tw of the preparations wh h w dehyd t d by the dry-chemical method discussed later served a tandard for those ruses

The Dry-Chemical Method of Dehydration Fresh virus infected immature leaves are collected and processed as quickly as possible With large leaves such as those of tobacco the mid ribs are removed and discarded The remaining tissue is cut in strips about 2 cm wide by means of shears These strips a e then clipped into short pieces of irregular size Grass leaves are clipped into lengths of about one half inch Fresh, granular $CaCl_2$ is quickly spread over the bottom of an aluminum baking pan which is 7 inches wide 11 inches long and 1½ inches deep The weight of the $CaCl_2$ used is double the weight of the water in the tissue The weight of water in succulent tissue such as tobac o leaves is figured on the basis of 95 per cent of the weight of the fresh tissue With less-succulent tis ue uch as gra s leaves the basis is 75 per cent

A wire screen support with the ends bent downward one-half inch is placed over the $CaCl_2$ and a cotton gauze is placed on top of the screen Not more

than 50 grams of the clipped tissue are *spread evenly over the* gauze A piece of window glass which is cut to fit the top of the pan, serves as a cover The edges of the glass and the pan are sealed by means of fresh, high grade, one inch zinc oxide surgical tape The pan is placed *in a refrigerator with the temperature* slightly above the freezing point

The tissue can be removed from the pans in five to seven days and placed in bottles that have been prepared in advance Two-ounce, *screw top bottles* with 24-mm openings are preferred These take No 5 rubber stoppers Stoppers are made of soft rubber that does not harden at temperatures near freezing These stoppers are superior to the types of screwcaps tested In each bottle is placed about 0 8 gram of reagent grade anhydrous magnesium perchlorate (Anhydrone) and another 0 8 gram wadded in a small square (3 by 3 inches) of Kleenex tissue paper brought together at the corners and wired tightly with soft copper or iron wire The bottles are tightly stoppered Two bottles usually accommodate the dried tissued from 50 grams of fresh leaves The wad of Anhydrone is removed with tweezers from the bottle just before the tissue is introduced The free granules of Anhydrone remain in the bottle, and the wad of Anhydrone is placed on top of the leaf tissue

The bottles are filled by means of an aluminum funnel with a shortened tip that is about 22 mm in diameter The gauze and leaf tissue are carefully lifted from the desiccating pan, and spread around the inside of the funnel cone With a spatula the tissue is removed from the gauze An aluminum rod, one fourth inch in diameter, is used to push the tissue out of the funnel and to pack the tissue in the bottle Aluminum is used to prevent static electricity trouble All operations are completed as rapidly as possible to *reduce moisture absorption by the dehydrated tissues and by the chemicals*

Anhydrone is more effective than CaCl for taking up traces of water and tests with the tobacco ringspot virus have shown that viability was extended *when the* $CaCl_2$ *was followed with Anhydrone* In order to obtain results quickly a test was conducted at 26 7 C Two uniform samples of ringspot leaf tissue had been dehydrated for 65 days over $CaCl_2$ at 1 1 C One of the preparations was continued with fresh $CaCl_2$ With the other, the $CaCl_2$ was replaced with fresh Anhydrone Both were continued at 1 1 C At the end of 25 days the desiccating agents were removed from both preparations which were then placed in tight containers, and kept at a temperature near 26 7°C for 31 days except that they were sampled for assays on the 15th day

The assays on the 15th day were made on 1250 cm^2 of tobacco leaves The preparation that had been processed with $CaCl_2$ induced 10 local lesions whereas the one processed with the Anhydrone, induced 248 local lesions Assays made the 31st day gave readings of 0 and 3 lesions from the respective preparations, each being tested on 670 cm^2 of tobacco leaves Another assay was made the 31st day on a sample from the $CaCl_2$ preparation, which had been held near 1 1 C throughout the entire period of the test This assay gave 1281 local lesions on 670 cm^2 of tobacco leaves

The advantages of Anhydrone and of *cool storage* temperature are clearly indicated in the results of these tests The activity of the tobacco ringspot

virus holds up remarkably well at 26 7 C however when the processing includes Anhydrone

Throughout these studies it has been observed that viruses are weakly active or inactive when moisture accidently enters the containers in an amount *sufficient to cause the tissues to lose the marked crispness characteristic of* properly dehydrated preparations

TABLE 2

THE PRESERVATION OF CERTAIN PLANT VIRUSES IN CLIPPED LEAF TISSUE DEHYDRATED BY MEANS OF CALCIUM CHLORIDE AND ANHYDROUS MAGNESIUM PERCHLORATE (ANHYDRONE)

Virus	Cult	Culture plant	Time stored		Activity
			Yrs	Mos	
Agropyron mosaic (Yellow mos strain)	163	Agropyron†	—	8	Weak
Alfalfa mosaic	57	Tobacco	5	6	Weak
Barley stripe mosaic (false stripe)	214	Wheat	—	8½	Strong
Brome mosaic	198	Brome‡	1	—	Strong
Celery mosaic (Southern)	3?	Tobacco	6	—	Weak
Cucumber mosaic (Doolittle)	27	Tobacco	6	—	Moderate
Oat mosaic (Apical strain)	37	Oats	2	8½	Very weak
Oat mosaic (Eyespot strain)	324	Oats	—	10½	Moderate
Potato veinbanding mosaic	*74*	*Tobacco*	*1*	*5*	*Moderate*
Potato veinbanding mosaic	74	Tobacco	2	2½	Inactive
Potato X mosaic (Common)	139	Datura§	4	4	Strong
Potato X mosaic (Ring pot type)	140	Tobacco	4	—	Strong
Potato X mosaic (Severe)	46	Tobacco	1	1½	Strong
Potato X mosaic (Severe)	46	Tobacco	3	4	Inactive
Potato X mosaic (Virulent)	93	Tobacco	4	7½	Strong
Tobacco etch	68	Datura§	4	—	Strong
Tobacco ringspot (Tobacco-Price)	101	Tobacco	4	3½	Strong
Tobacco ringspot (Soybean Allington)	130	Tobacco	4	1½	Strong
Tobacco streak	208	Tobacco	—	14	Strong
Turnip mosaic	*249*	*Turnip*	*2*	*3*	*Very weak*
Wheat mosaic rosette (Soil borne type)	11	Wheat	3	½	*Very weak*
Wheat streak mosaic	199	Wheat	2	2½	Moderate
Wheat yellow mosaic (Soil borne type)	13	Wheat	3	8½	Moderate

Dehydration and storage were at temperatures ranging slightly above and below freezing The regulators ... household refrigerators and ice-cream storage cabinets ... as possible for 1.0 C. When the cooling ... were defrosted the temperatures at times were slightly below freezing and as frost collected on the coils the temperatures sometimes reached 4 C.

† Ag pyr pens

‡ B mus nerm

§ D ... mon m

TABLE 2 lists a number of viruses that are being carried in dehydrated leaf tissue In addition to these many tests indicate that the viruses of turnip mosaic the oat mosaics and wheat mosaic rosette are especially difficult to preserve by dry chemical dehydration It is possible that higher initial titers of these viruses may be obtained in more susceptible host plants than those used. It is also quite possible that the preservation time of all viruses would be extended if the tissues were dehydrated *in the absence of oxygen* and if storage of the dehydrated preparations were at subfreezing temperatures

Although phosphorus pentoxide removes traces of water more completely

than does Anhydrone, no safe and convenient way has been found to use this very dangerous chemical Activated alumina, color indicator grade may have some advantages over Anhydrone On the other hand, the results with the present method of dry-chemical dehydration *in situ* show that with very modest facilities it is possible to maintain several of the relatively unstable viruses with much less effort and expense than is possible by the method of continuous culture in growing plants Dehydrated preparations of cucumber mosaic virus tobacco ringspot virus and tobacco streak virus have been transported by railway mail within the United States, and all remained viable

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GENERAL DISCUSSION OF VIRUS NOMENCLATURE

By Sir MacFarlane Burnet
The Walter and Eliza Hall Institute for Medical Research Melbourne Australia

It seems that the participants in the Conference on which this monograph is based generally agreed that a satisfactory classification is desirable but were also very conscious that there are important gaps in the knowledge required for such a classification

The attendance at the Conference was almost fully representative of virologists from the United States and Canada and there was a sufficient representation from other parts of the world to justify considerable weight being attached to the conclusions and attitudes It is therefore desirable that any smaller groups or individuals presenting taxonomic proposals in the virus field should bear them in mind We should ask specifically that (1) in any revision of the Bergey classification of viruses full use be made of the work of the sectional subcommittees which were appointed to deal with certain groups of viruses at the Rio congress and which are expected to report at the Rome meeting in 1953 and that (2) those virologists who will be present at the Rome meeting be guided not only by the reports of the subcommittees but also by the publications arising from this Conference

I believe that the *opinion of the majority* favors the view that Linnean binomials of definitive status *can* be applied to certain groups of viruses either now or in the immediate future It is also evident that definitive names should not yet be adopted for many viruses Any names given those viruses should carry some indication that the names are provisional only These conclusions seem to be clearly applicable to the animal viruses and with somewhat less clarity to the plant viruses Dr Adams s paper indicated that when the time is ripe bacterial viruses may be found divisible into species that can be rigidly defined by a combination of serological characterization and capacity to produce multiple infections Until wider systematic study can be made however there is insufficient material on which to suggest the appropriate criteria for the definition of genera No action is called for beyond the accumulation of relevant experimental data

My general impressions of the more specific problems of the animal viruses are

(1) There is substantial agreement in regard to the classification of the Rickettsiae proper and for this group a valid nomenclature can be regarded as having been initiated by da Rocha Lima

(2) One must have much sympathy with the view that viruses of the psittacosis group are more closely related to the rickettsiae than to the more typical viruses Nevertheless this group was one of those chosen for study by the Rio congress and it is desirable that the question of its nomenclature be left open until a decision is given at the Rome congress

(3) There seems to be general agreement that the mechanism developed at Rio (the appointment of small subcommittees to make recommendations in regard to particular groups) is functioning with reasonable adequacy It

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